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RECOMBINANT HTLV-III PROTEINS AND USES THEREOF

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(56) Prior Art Documents
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(57) Claim

1. A process for stimulating a lymphocyte proliferative response in humans which comprises treating humans in need of stimulation of a lymphocyte proliferative response with a recombinant HIV portion of an HTLV-III protein selected from the group consisting of R10, PB1, 590 and KH1, wherein members of said group are as hereinbefore described.

FORM 1

SPRUSON & FERGUSON

61394 1

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

Repligen Corporation, of One Kendall Square, Cambridge, Massachusetts, 02139, UNITED STATES OF AMERICA, hereby apply for the grant of a standard patent for an invention entitled:

Recombinant HTLV-III Proteins and Uses Thereof

which is described in the accompanying complete specification.

Details of basic application(s):-

Basic Applic. No:	Country:	Application Date:
107231	US	9 October 1987

The address for service is:-

Spruson & Ferguson
Patent Attorneys
Level 33 St Martins Tower
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Sydney New South Wales Australia

DATED this EIGHTEENTH day of AUGUST 1988

Repligen Corporation

By: M J Ferguson

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
OUR REF: 56873
S&F CODE: 61175

5845/4

Spruson & Ferguson

AUSTRALIA
CONVENTION
STANDARD
& PETTY PATENT
DECLARATION
SFP4

COMMONWEALTH OF AUSTRALIA

THE PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made for a patent for an invention entitled:

Title of Invention: Recombinant HTLV-III Proteins and Uses Thereof

I/~~We~~ Thomas H. Fraser

Full name(s) and address(es) of Declarant(s)

of Repligen Corporation
One Kendall Square
Building 700
Cambridge, Massachusetts 02139 USA

do solemnly and sincerely declare as follows:–

Full name(s) of Applicant(s)

1. ~~I am/We are the applicant(s) for the patent~~

(or, in the case of an application by a body corporate)

1. I am/~~We are~~ authorised by Repligen Corporation

~~the applicant(s) for the patent to make this declaration on its/their behalf~~

2. The basic application~~(s)~~ as defined by Section 141 of the Act was/~~were~~ made

Basic Country(ies) in The United States of America U.S. Ser. No. 107,231

Priority Date(s) on October 9, 1987

Basic Applicant(s) by Scott D. Putney, Debra Lynn, Kashayar Javaherian, William T. Mueller, and John Farley

3. ~~I am/We are the actual inventor(s) of the invention referred to in the basic application(s)~~

Full name(s) and address(es) of inventor(s)

(or where a person other than the inventor is the applicant)

3. Scott D. Putney, Debra Lynn, Kashayar Javaherian, William T. Mueller, and John Farley

of 5 Epping St., Arlington, MA 02174 USA; 11 Allen St., Apt. 11, Arlington MA 02174 USA; 27 Webster Rd., Lexington, MA 02173 USA; 26 Copeland St., Watertown, MA 02172 USA; and 261 Culver Rd., #9, Rochester, NY 14607 US (respectively)

~~is~~/are the actual inventor(s) of the invention and the facts upon which the applicant~~(s)~~ is/~~are~~ entitled to make the application are as follows:

Set out how Applicant(s) derive title from actual inventor(s) e.g. The Applicant(s) is/are the assignee(s) of the invention from the inventor(s)

The Applicant is the assignee of the invention from the inventors.

4. The basic application~~(s)~~ referred to in paragraph 2 of this Declaration was/~~were~~ the first application~~(s)~~ made in a Convention country in respect of the invention ~~(s)~~ the subject of the application.

Declared at Cambridge, MA this 21st day of April 1988

X T.H. Fraser
Signature of Declarant(s)
Thomas H. Fraser, Executive Vice President
Repligen Corporation

SFP4 To: The Commissioner of Patents

S & F Ref: 56873

FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

613944

(ORIGINAL)

FOR OFFICE USE:

Class Int Class

Complete Specification Lodged:
Accepted:
Published:

Priority:

Related Art:

Name and Address of Applicant:

Repligen Corporation
One Kendall Square
Cambridge Massachusetts 02139
UNITED STATES OF AMERICA

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Level 33 St Martins Tower, 31 Market Street
Sydney, New South Wales, 2000, Australia

Complete Specification for the invention entitled:

Recombinant HTLV-III Proteins and Uses Thereof

The following statement is a full description of this invention, including the best method of performing it known to me/us

5845/3

ABSTRACT

A process for stimulating a lymphocyte proliferative response in humans which comprises treating humans in need of stimulation of a lymphocyte proliferative response with a recombinant HIV portion of an HTLV-III protein selected from the group consisting of R10, PB1, 590 and KH1, wherein members of said group are as hereinbefore described.

DESCRIPTION

RECOMBINANT HTLV-III PROTEINS AND USES THEREOF

Background of the Invention

Human T-cell lymphotropic virus (HTLV-III), lymphadenopathy-associated virus (LAV), or AIDS-associated retrovirus (ARV) has been identified as the cause of acquired immune deficiency syndrome (AIDS) (Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C., [1984] Science 224:497-500). The virus displays tropism for the $OKT4^+$ lymphocyte subset (Klatzmann, D., Barre-Sinoussi, F., Nugeyre, M.T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vezinet, F., Rouzioux, C., Gluckman, J.C., Chermann, J.C. and Montagnier, L. [1984] Science 225:59-63). Antibodies against HTLV-III proteins in the sera of most AIDS and AIDS related complex (ARC) patients, and in asymptomatic people infected with the virus (Sarngadharan, M.G., Popovic, M., Bruch, L., Schupbach, J. and Gallo, R.C. [1984] Science 224:506-508) have made possible the development of immunologically based tests that detect antibodies to these antigens. These tests are used to limit the spread of HTLV-III through blood transfusion by identifying blood samples of people infected with the virus. Diagnostic tests currently available commercially use the proteins of inactivated virus as antigens.

In addition to allowing new approaches for diagnosis, recombinant DNA holds great promise for the development of vaccines against both bacteria and viruses (Wilson, T. [1984] Bio/Technology 2:29-39). The most widely employed organisms to express recombinant vaccines have been E. coli, S. cerevisiae and cultured mammalian cells. For example, subunit vaccines against foot and mouth disease (Kleid, D.G., Yansura, D., Small, B., Dowbenko, D., Moore, D.M., Brubman, M.J., McKercher, P.D., Morgan, D.O., Robertson, B.H. and Bachrach, H.L. [1981] Science 214:1125-1129) and malaria (Young, J.F., Hockmeyer, W.T., Gross, M., Ripley Ballou, W., Wirtz, R.A., Trosper, J.H., Beaudoin, R.L., Hollingdale, M.R., Miller, L.M., Diggs, C.L. and Rosenberg, M. [1985] Science 228:958-962) have been synthesized in E. coli. Other examples are hepatitis B surface antigen produced in yeast (McAleer, W.J., Buynak, E.B., Maigetter, R.Z., Wampler, D.E., Miller, W.J. and Hilleman, M.R. [1984] Nature 307: 178-180) and a herpes vaccine produced in mammalian cells (Berman, P.W., Gregory, T., Chase, D. and Lasky, L.A. [1984] Science 227:1490-1492).

There is a real need at this time to develop a vaccine for AIDS. No such vaccine is known to exist.

Brief Summary of the Invention

The subject invention concerns novel recombinant HTLV-III proteins and the uses thereof. More specifically, the subject invention concerns novel recombinant HTLV-III envelope proteins which can be used in the diagnosis, prophylaxis or therapy of AIDS. Further, the recombinant HTLV-III envelope protein

fragments of the invention can be used to stimulate a lymphocyte proliferative response in HTLV-III infected humans. This then would stimulate the immune system to respond to HTLV-III in such individuals and, therefore, the envelope protein fragments can provide protection and be of therapeutic value. These novel proteins are encoded on bacterial plasmids which can be used to transform suitable hosts, for example, E. coli, using standard procedures.

Reference to the Drawings

FIGURE 1--This is a flow chart of the construction of plasmid pREV2.2 which is used to construct vectors encoding novel proteins.

FIGURE 2--This is a diagram of plasmid pREV2.2 showing the multiple cloning site.

FIGURE 3--This is a schematic of the HTLV-III envelope gene and the novel recombinant proteins obtained therefrom.

FIGURE 4--Drawing showing the removal of N-terminal non-HTLV-III sequences of PB1.

FIGURE 5--Drawing showing the removal of C-terminal non-HTLV-III sequences from PB1.

Detailed Disclosure of the Invention

Expression vector plasmid pREV2.2 was constructed from plasmid pBG1. The flow chart showing the construction of this plasmid is given in Figure 1 of the drawings.

Plasmid pR10 contains approximately 1275 base pairs of DNA encoding the HTLV-III env gene from essentially

the KpnI site to the BglII site. This plasmid in a suitable bacterial host, e.g., E. coli, can be used to produce the novel recombinant HTLV-III 95 kD fusion protein denoted R10. The amino acid sequence of fusion protein R10 is shown in Table 8; the DNA sequence encoding this protein is shown in Table 8A. The amino acid sequence of the HIV portion of protein R10 is shown in Table 12. The DNA sequence encoding the HIV portion of protein R10 is shown in Table 12A.

Plasmid pPB1 contains approximately 540 base pairs of DNA encoding essentially the HTLV-III env gene from the PvuII site to the BglII site. This plasmid in a suitable host, e.g., E. coli, can be used to produce the novel recombinant HTLV-III 26 kD fusion protein denoted PB1. The amino acid sequence of fusion protein PB1 is shown in Table 9; the DNA sequence encoding this protein is shown in Table 9A. The amino acid sequence of the HIV portion of protein PB1 is shown in Table 13. The DNA sequence encoding the HIV portion of protein PB1 is shown in Table 13A.

Plasmid p590 contains approximately 1055 base pairs of DNA encoding essentially the HTLV-III env gene from the PvuII site to the HindIII site. This plasmid in a suitable host, e.g., E. coli, can be used to produce the novel recombinant HTLV-III 86 kD protein denoted 590. The amino acid sequence of fusion protein 590 is shown in Table 10; the DNA sequence encoding this protein is shown in Table 10A. The amino acid sequence of the HIV portion of protein 590 is shown in Table 14. The DNA sequence encoding the HIV portion of protein 590 is shown in Table 14A.

Plasmid pKH1 contains approximately 1830 base pairs of DNA encoding essentially the HTLV-III env gene from the KpnI site to the HindIII site. This plasmid in a suitable host, e.g., E. coli, can be used to produce the novel recombinant HTLV-III 70 kD protein denoted KH1. The amino acid sequence of fusion protein KH1 is shown in Table 11; the DNA sequence encoding this protein is shown in Table 11A. The amino acid sequence of the HIV portion of protein KH1 is shown in Table 15. The DNA sequence encoding the HIV portion of protein KH1 is shown in Table 15A.

Plasmid pBG1 is deposited in the E. coli host MS371 with the Northern Regional Research Laboratory (NRRL), U.S. Department of Agriculture, Peoria, Illinois, USA. It is in the permanent collection of this repository. E. coli MS371(pBG1), NRRL B-15904, was deposited on Nov. 1, 1984. E. coli MS371, NRRL B-15129 is now available to the public. E. coli SG20251, NRRL B-15918, was deposited on Dec. 12, 1984. NRRL B-15904 and NRRL B-15918 will be available to the public upon the grant of a patent which discloses them. Other cultures which were deposited with NRRL and their deposit dates and numbers are as follows:

Culture	Repository No.	Date of Deposit
E. coli JM103(pREV2.2)	NRRL B-18091	July 30, 1986
E. coli SG20251(pR10)	NRRL B-18093	July 30, 1986
E. coli SG20251(pPB1)	NRRL B-18092	July 30, 1986
E. coli SG20251(p590)	NRRL B-18094	July 30, 1986
E. coli CAG629(pKH1)	NRRL B-18095	July 30, 1986

The above deposits will be maintained in the NRRL repository for at least 30 years and will be made available to the public upon the grant of a patent disclosing them. The deposits are also available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

The novel HTLV-III proteins of the subject invention can be expressed in Saccharomyces cerevisiae using plasmids containing the inducible galactose promoter from this organism (Broach, J.R., Li, Y., Wu, L.C. and Jayaram, M. in Experimental Manipulation of Gene Expression [1983] p. 83, ed. M. Inouye, Academic Press). These plasmids are called YEp51 and YEp52 (Broach, J.R. et al. [1983]) and contain the E. coli origin of replication, the gene for β-lactamase, the yeast LEU2 gene, the 2 μm origin of replication and the 2 μm circle REP3 locus. Recombinant gene expression is driven by the yeast GAL10 gene promoter.

Yeast promoters such as galactose and alcohol dehydrogenase (Bennetzen, J.L. and Hall, B.D. [1982] J. Biol. Chem. 257:3018; Ammerer, G. in Methods in Enzymology [1983] Vol. 101, p. 192), phosphoglycerate kinase (Derynck, R., Hitzeman, R.A., Gray, P.W., Goeddel, D.V., in Experimental Manipulation of Gene Expression [1983] p. 247, ed. M. Inouye, Academic Press), triose phosphate isomerase (Alber, T. and Kawasaki, G. [1982] J. Molec. and Applied Genet. 1:419), or enolase (Innes, M.A. et al. [1985] Science 226:21) can be used.

The genes disclosed herein can be expressed in simian cells. When the genes encoding these proteins are cloned into one of the plasmids as described in Okayama and Berg (Okayama, H. and Berg, P. [1983] Molec. and Cell. Biol. 3:280) and references therein, or COS cells transformed with these plasmids, synthesis of HTLV-III proteins can be detected immunologically.

Other mammalian cell gene expression/protein production systems can be used. Examples of other such systems are the vaccinia virus expression system (Moss, B. [1985] Immunology Today 6:243; Chakrabarti, S., Brechling, K., Moss, B. [1985] Molec. and Cell. Biol. 5:3403) and the vectors derived from murine retroviruses (Mulligan, R.C. in Experimental Manipulation of Gene Expression [1983] p. 155, ed. M. Inouye, Academic Press).

The HTLV-III proteins of the subject invention can be chemically synthesized by solid phase peptide synthetic techinques such as BOC and FMOC (Merrifield, R.B. [1963] J. Amer. Chem. Soc. 85:2149; Chang, C. and Meienhofer, J. [1978] Int. J. Peptide Protein Res. 11:246).

As is well known in the art, the amino acid sequence of a protein is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins, different nucleotide sequences can code for a particular amino acid. Thus, the genetic code can be depicted as follows:

	Phenylalanine (Phe)	TTK	Histidine (His)	CAK
	Leucine (Leu)	XTY	Glutamine (Gln)	CAJ
	Isoleucine (Ile)	ATM	Asparagine (Asn)	AAK
	Methionine (Met)	ATG	Lysine (Lys)	AAJ
5	Valine (Val)	GTL	Aspartic acid (Asp)	GAK
	Serine (Ser)	QRS	Glutamic acid (Glu)	CAJ
	Proline (Pro)	CCL	Cysteine (Cys)	TGK
	Threonine (Thr)	ACL	Tryptophan (Trp)	TGG
	Alanine (Ala)	GCL	Arginine (Arg)	WGZ
10	Tyrosine (Tyr)	TAK	Glycine (Gly)	GGL
	Termination Signal	TAJ		
	Termination Signal	TGA		

Key: Each 3-letter deoxynucleotide triplet corresponds to a trinucleotide of mRNA, having a 5'-end on the left and a 3'-end on the right. All DNA sequences given herein are those of the strand whose sequence corresponds to the mRNA sequence, with thymine substituted for uracil. The letters stand for the purine or pyrimidine bases forming the deoxynucleotide sequence.

A = adenine
G = guanine
C = cytosine
T = thymine
X = T or C if Y is A or G
X = C if Y is C or T
Y = A, G, C or T if X is C
Y = A or G if X is T
W = C or A if Z is A or G
W = C if Z is C or T
Z = A, G, C or T if W is C
Z = A or G if W is A
QR = TC if S is A, G, C or T; alternatively QR = AG if S is T or C

J = A or G
K = T or C
L = A, T, C or G
M = A, C or T

The above shows that the novel amino acid sequences of the HTLV-III proteins of the subject invention can be prepared by nucleotide sequences other than those disclosed herein. Functionally equivalent nucleotide sequences encoding the novel amino acid sequences of these HTLV-III proteins, or fragments thereof having HTLV-III antigenic or immunogenic or therapeutic activity, can be prepared by known synthetic procedures. Accordingly, the subject invention includes such functionally equivalent nucleotide sequences.

Thus the scope of the subject invention includes not only the specific nucleotide sequences depicted herein, but also all equivalent nucleotide sequences coding for molecules with substantially the same HTLV-III antigenic or immunogenic or therapeutic activity.

Further, the scope of the subject invention is intended to cover not only the specific amino acid sequences disclosed, but also similar sequences coding for proteins or protein fragments having comparable ability to induce the formation of and/or bind to specific HTLV-III antibodies.

The term "equivalent" is being used in its ordinary patent usage here as denoting a nucleotide sequence which performs substantially as the nucleotide sequence identified herein to produce molecules with substantially the same HTLV-III antigenic or immunogenic or therapeutic activity in essentially the same kind of hosts. Within this definition are subfragments which have HTLV-III antigenic or immunogenic or therapeutic activity.

As disclosed above, it is well within the skill of those in the genetic engineering art to use the nucleotide sequences encoding HTLV-III antigenic or immunogenic or therapeutic activity of the subject invention to produce HTLV-III proteins via microbial processes. Fusing the sequences into an expression vector and transforming or transfecting into hosts, either eukaryotic (yeast or mammalian cells) or prokaryotic (bacterial cells), are standard procedures used in producing other well-known proteins, e.g., insulin, interferons, human growth hormone, IL-1, IL-2, and the like. Similar procedures, or obvious modifications thereof, can be employed to prepare HTLV-III proteins by microbial means or tissue-culture technology in accord with the subject invention.

The nucleotide sequences disclosed herein can be prepared by a "gene machine" by procedures well known in the art. This is possible because of the disclosure of the nucleotide sequence.

The restriction enzymes disclosed can be purchased from Bethesda Research Laboratories, Gaithersburg, MD, or New England Biolabs, Beverly, MA. The enzymes are used according to the instructions provided by the supplier.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. These procedures are all described in Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Thus, it is within the skill of those in the genetic engineering

art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, e.g., E. coli cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

Immunochemical assays employing the HTLV-III proteins of the invention can take a variety of forms. The preferred type is a solid phase immunometric assay. In assays of this type, an HTLV-III protein is immobilized on a solid phase to form an antigen-immunoadsorbent. The immunoadsorbent is incubated with the sample to be tested. After an appropriate incubation period, the immunoadsorbent is separated from the sample and labeled anti-(human IgG) antibody is used to detect human anti-HTLV-III antibody bound to the immunoadsorbent. The amount of label associated with the immunoadsorbent can be compared to positive and negative controls to assess the presence or absence of anti-HTLV-III antibody.

The immunoadsorbent can be prepared by adsorbing or coupling a purified HTLV-III protein to a solid phase. Various solid phases can be used, such as beads formed of glass, polystyrene, polypropylene, dextran or other material. Other suitable solid phases include tubes or plates formed from or coated with these materials.

The HTLV-III proteins can be either covalently or non-covalently bound to the solid phase by techniques such as covalent bonding via an amide or ester linkage or adsorption. After the HTLV-III protein is affixed to the solid phase, the solid phase can be post-coated with an animal protein, e.g., 3% fish gelatin. This provides a blocking protein which reduces nonspecific

adsorption of protein to the immunoadsorbent surface.

The immunoadsorbent is then incubated with the sample to be tested for anti-HTLV-III antibody. In blood screening, blood plasma or serum is used. The plasma or serum is diluted with normal animal plasma or serum. The diluent plasma or serum is derived from the same animal species that is the source of the anti-(human IgG) antibody. The preferred anti-(human IgG) antibody is goat anti-(human IgC) antibody. Thus, in the preferred format, the diluent would be goat serum or plasma.

The conditions of incubation, e.g., pH and temperature, and the duration of incubation are not crucial. These parameters can be optimized by routine experimentation. Generally, the incubation will be run for 1-2 hr at about 45°C in a buffer of pH 7-8.

After incubation, the immunoadsorbent and the sample are separated. Separation can be accomplished by any conventional separation technique such as sedimentation or centrifugation. The immunoadsorbent then may be washed free of sample to eliminate any interfering substances.

The immunoadsorbent is incubated with the labeled anti-(human IgG) antibody (tracer) to detect human antibody bound thereto. Generally the immunoadsorbent is incubated with a solution of the labeled anti-(human IgG) antibody which contains a small amount (about 1%) of the serum or plasma of the animal species which serves as the source of the anti-(human IgG) antibody. Anti-(human IgG) antibody can be obtained from any animal source. However, goat anti-(human IgG) antibody is preferred. The anti-(human IgG) antibody can be an

antibody against the Fc fragment of human IgG, for example, goat anti-(human IgG) Fc antibody.

The anti-(human IgG) antibody or anti-(human IgG)Fc can be labeled with a radioactive material such as ^{125}iodine; labeled with an optical label, such as a fluorescent material; or labeled with an enzyme such as horseradish peroxidase. The anti-human antibody can also be biotinylated and labeled avidin used to detect its binding to the immunoadsorbent.

After incubation with the labeled antibody, the immunoadsorbent is separated from the solution and the label associated with the immunoadsorbent is evaluated. Depending upon the choice of label, the evaluation can be done in a variety of ways. The label may be detected by a gamma counter if the label is a radioactive gamma emitter, or by a fluorimeter, if the label is a fluorescent material. In the case of an enzyme, label detection may be done colorimetrically employing a substrate for the enzyme.

The amount of label associated with the immunoadsorbent is compared with positive and negative controls in order to determine the presence of anti-HTLV-III antibody. The controls are generally run concomitantly with the sample to be tested. A positive control is a serum containing antibody against HTLV-III; a negative control is a serum from healthy individuals which does not contain antibody against HTLV-III.

For convenience and standardization, reagents for the performance of the immunometric assay can be assembled in assay kits. A kit for screening blood, for example, can include:

(a) an immunoadsorbent, e.g., a polystyrene bead coated with an HTLV-III protein;

(b) a diluent for the serum or plasma sample, e.g., normal goat serum or plasma;

(c) an anti-(human IgG) antibody, e.g., goat anti-(human IgG) antibody in buffered, aqueous solution containing about 1% goat serum or plasma;

(d) a positive control, e.g., serum containing antibody against at least one of the novel HTLV-III proteins; and

(e) a negative control, e.g., pooled sera from healthy individuals which does not contain antibody against at least one of the novel HTLV-III proteins.

If the label is an enzyme, an additional element of the kit can be the substrate for the enzyme.

Another type of assay for anti-HTLV-III antibody is an antigen sandwich assay. In this assay, a labeled HTLV-III protein is used in place of anti-(human IgG) antibody to detect anti-HTLV-III antibody bound to the immunoadsorbent. The assay is based in principle on the bivalency of antibody molecules. One binding site of the antibody binds the antigen affixed to the solid phase; the second is available for binding the labeled antigen. The assay procedure is essentially the same as described for the immunometric assay except that after incubation with the sample, the immunoadsorbent is incubated with a solution of labeled HTLV-III protein. HTLV-III proteins can be labeled with radioisotope, an enzyme, etc. for this type of assay.

In a third format, the bacterial protein, protein A, which binds the Fc segment of an IgG molecule without interfering with the antigen-antibody interaction can be used as the labeled tracer to detect anti-HTLV-antibody adsorbed to the immunoadsorbent. Protein A

can be readily labeled with a radioisotope, enzyme or other detectable species.

Immunochemical assays employing an HTLV-III protein have several advantages over those employing a whole (or disrupted) virus. Assays based upon an HTLV-III protein will alleviate the concern over growing large quantities of infectious virus and the inherent variability associated with cell culturing and virus production. Further, the assay will help mitigate the real or perceived fear of contracting AIDS by technicians in hospitals, clinics and blood banks who perform the test.

Vaccines comprising one or more of the HTLV-III proteins, disclosed herein, and variants thereof having antigenic properties, can be prepared by procedures well known in the art. For example, such vaccines can be prepared as injectables, e.g., liquid solutions or suspensions. Solid forms for solution in, or suspension in, a liquid prior to injection also can be prepared. Optionally, the preparation also can be emulsified. The active antigenic ingredient or ingredients can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Examples of suitable excipients are water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants, which enhance the effectiveness of the vaccine. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations.

For suppositories, traditional binders and carriers include, for example, polyalkalene glycols or triglycerides. Suppositories can be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%, preferably about 1 to about 2%. Oral formulations can include such normally employed excipients as, for example, pharmaceutical grades of manitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain from about 10% to about 95% of active ingredient, preferably from about 25% to about 70%.

The proteins can be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required

to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosage ranges are of the order of about several hundred micrograms active ingredient per individual. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in one or two week intervals by a subsequent injection or other administration.

HTLV-III is known to undergo amino acid sequence variation, particularly in the envelope gene (Starcich, B.R. [1986] Cell 45:637-648; Hahn, B.H. et al. [1986] Science 232:1548-1553). Over 100 variants have been analyzed by molecular cloning and restriction enzyme recognition analysis, and several of these have been analyzed by nucleotide sequencing. Some of these are the HTLV-III isolates known as RF (Popovic, M. et al. [1984] Science 224:497-500), WMJ-1 (Hahn, B.H. et al. [1986] Science 232:1548-1553), LAV (Wain-Hobson, S. et al. [1985] Cell 40:9-17), and ARV-2 (Sanchez-Pescador, R. et al. [1985] Science 227:484-492). Although the subject invention describes the sequence from one HTLV-III isolate, the appropriate envelope regions of any HTLV-III isolate can be produced using the procedures described herein for preparing R10, PB1, 590, and KH1. The HTLV-III proteins from different viral isolates can be used in vaccine preparations, as disclosed above, to protect against infections by different HTLV-III isolates. Further, a vaccine preparation can be

made using more than one recombinant antigenic protein from more than one HTLV-III isolate to provide immunity and thus give better protection against AIDS.

Following are examples which illustrate the process of the invention, including the best mode. These examples should not be construed as limiting. All solvent mixture proportions are by volume unless otherwise noted.

Example 1--Construction of plasmid pREV2.2

The pREV2.2 plasmid expression vector was constructed from plasmid pBG1. Plasmid pBG1 can be isolated from its E. coli host by well known procedures, e.g., using cleared lysate-isopycnic density gradient procedures, and the like. Like pBG1, pREV2.2 expresses inserted genes behind the E. coli promoter. The differences between pBG1 and pREV2.2 are the following:

1. pREV2.2 lacks a functional replication of plasmid (rop) protein.
2. pREV2.2 has the trpA transcription terminator inserted into the AatII site. This sequence insures transcription termination of over-expressed genes.

3. pREV2.2 has genes to provide resistance to ampicillin and chloramphenicol, whereas pBG1 provides resistance only to ampicillin.
4. pREV2.2 contains a sequence encoding sites for several restriction endonucleases.

The following procedures, shown in Figure 1 of the drawings, were used to make each of the four changes listed above:

1a. 5 µg of plasmid pBG1 was restricted with NdeI which gives two fragments of approximately 2160 and 3440 base pairs.

1b. 0.1 µg of DNA from the digestion mixture, after inactivation of the NdeI, was treated with T4 DNA ligase under conditions that favor intramolecular ligation (200 µl reaction volume using standard T4 ligase reaction conditions [New England Biolabs, Beverly, MA]). Intramolecular ligation of the 3440 base pair fragment gave an ampicillin resistant plasmid. The ligation mixture was transformed into the recipient strain E. coli JM103 (available from New England Biolabs) and ampicillin resistant clones were selected by standard procedures.

1c. The product plasmid, pBG1ΔN, where the 2160 base pair NdeI fragment is deleted from pBG1, was selected by preparing plasmid from ampicillin resistant clones and determining

the restriction digestion patterns with NdeI and SalI (product fragments approximately 1790 and 1650). This deletion inactivates the rop gene that controls plasmid replication.

2a. 5 µg of pBG1ΔN was then digested with EcoRI and BclI and the larger fragment, approximately 2455 base pairs, was isolated.

2b. A synthetic double stranded fragment was prepared by the procedure of Itakura et al. (Itakura, K., Rossi, J.J. and Wallace, R.B. [1984] Ann. Rev. Biochem. 53:323-356, and references therein) with the structure shown in Table 1. This fragment has BclI and EcoRI sticky ends and contains recognition sequences for several restriction endonucleases.

2c. 0.1 µg of the 2455 base pair EcoRI-BclI fragment and 0.01 µg of the synthetic fragment were joined with T4 DNA ligase and competent cells of strain JM103 were transformed. Cells harboring the recombinant plasmid, where the synthetic fragment was inserted into pBG1ΔN between the BclI and EcoRI sites, were selected by digestion of the plasmid with HpaI and EcoRI. The diagnostic fragment sizes are approximately 2355 and 200 base pairs. This plasmid is called pREV1.

2d. 5 µg of pREV1 were digested with AatII, which cleaves uniquely.

2e. The double stranded fragment shown in Table 2 was synthesized. This fragment has AatII sticky ends and contains the trpA transcription termination sequence.

2f. 0.1 µg of AatII digested pREV1 was ligated with 0.01 µg of the synthetic fragment in a volume

of 20 µl using T4 DNA ligase.

2g. Cells of strain JM103, made competent, were transformed and ampicillin resistant clones selected.

2h. Using a KpnI, EcoRI double restriction digest of plasmid isolated from selected colonies, a cell containing the correct construction was isolated. The sizes of the KpnI, EcoRI generated fragments are approximately 2475 and 80 base pairs. This plasmid is called pREV1TT and contains the trpA transcription terminator.

3a. 5 µg of pREV1TT, prepared as disclosed above (by standard methods) was cleaved with NdeI and XmnI and the approximately 850 base pair fragment was isolated.

3b. 5 µg of plasmid pBR325 (BRL, Gaithersburg, MD), which contains the genes conferring resistance to chloramphenicol as well as to ampicillin and tetracycline, was cleaved with BclI and the ends blunted with Klenow polymerase and deoxynucleotides. After inactivating the enzyme, the mixture was treated with NdeI and the approximately 3185 base pair fragment was isolated. This fragment contains the genes for chloramphenicol and ampicillin resistance and the origin of replication.

3c. 0.1 µg of the NdeI-XmnI fragment from pREV1TT and the NdeI-BclI fragment from pBR325 were ligated in 20 µl with T4 DNA ligase and the mixture used to transform competent cells of strain JM103. Cells resistant to both ampicillin and chloramphenicol were selected.

3d. Using an EcoRI and NdeI double digest of plasmid from selected clones, a plasmid was selected giving fragment sizes of approximately 2480, 1145, and 410 base pairs. This is called plasmid pREV1TT/chl and has genes for resistance to both ampicillin and chloramphenicol.

4a. A double stranded fragment shown in Table 3 was synthesized. This fragment, with a blunt end and an SstI sticky end, contains recognition sequences for several restriction enzyme sites.

4b. 5 µg of pREV1TT/chl was cleaved with NruI (which cleaves about 20 nucleotides from the BclI site) and SstI (which cleaves within the multiple cloning site). The larger fragment, approximately 3990 base pairs, was isolated from an agarose gel.

4c. 0.1 µg of the NruI-SstI fragment from pREV1TT/chl and 0.01 µg of the synthetic fragment were treated with T4 DNA ligase in a volume of 20 µl.

4d. This mixture was transformed into strain JM103 and ampicillin resistant clones were selected.

4e. Plasmid was purified from several clones and screened by digestion with MluI or ClaI. Recombinant clones with the new multiple cloning site will give one fragment when digested with either of these enzymes, because each cleaves the plasmid once.

4f. The sequence of the multiple cloning site was verified. This was done by restricting the

plasmid with HpaI and PvuII and isolating the 1395 base pair fragment, cloning it into the SmaI site of mp18 and sequencing it by dideoxynucleotide sequencing using standard methods.

4g. This plasmid, called pREV2.2 is diagrammed in Figure 2 of the drawings.

Example 2--Construction of and expression from pR10

Plasmid pR10, which contains approximately 1275 base pairs of DNA encoding the HTLV-III env gene from essentially the KpnI site to the BglII site, and from which is synthesized an approximately 95 kD fusion protein containing this portion of the gp120 envelope protein, can be constructed as follows:

1. Synthesizing the DNA with the sequence shown in Table 4. This DNA fragment can be synthesized by standard methods (Itakura, et al., supra, and references therein) and encodes a portion of gp120. It has a blunt end on the 5' end and an end which will ligate with a BamHI overhang on the 3' end.
2. Restricting 5 ug of plasmid pBG1 with BclI, filling in the overhanging ends with Klenow polymerase and deoxyribonucleotide triphosphates (dNTPs), restricting this fragment with BamHI and isolating the large fragment, approximately 8.9 kb, from an agarose gel.
3. Ligating 0.1 ug of the fragment in Table 4 with 0.1 ug of the pBG1 fragment in a volume of 20 µl using T4 DNA ligase, transforming the ligation mixture into competent cell strain SG20251 (Gottesman, S., Halpern, E. and Trisler, P. [1981] Journal of Bacteriology 148:265-273), and selecting ampicillin resistant transformants.

4. Selecting, using the AhaIII restriction pattern of purified plasmid, cells harboring the recombinant plasmid with the synthesized fragment in the orientation whereby the fragment blunt end ligated to the pBG1 fragment filled-in BclI end and the BamHI overhanging ends ligated together. AhaIII digestion of the proper plasmid gives fragment lengths of approximately 5300, 3170, 690, 640, 330, and 20 base pairs.
5. When the strain harboring this recombinant plasmid is grown in 2% medium (2% yeast extract, bactotryptone, casamino acids (Difco, Detroit, MI), 0.2% potassium monobasic, 0.2% potassium dibasic, and 0.2% sodium dibasic) containing 50 µg/ml ampicillin and the total complement of cellular proteins electrophoresed on an SDS-polyacrylamide gel, a prominent protein of approximately 95 kD can be visualized by either coomassie blue staining or by western blot analysis using as probe selected sera from AIDS, ARC, or HTLV-III infected individuals.

Example 3--Purification of recombinant protein containing HTLV-III envelope sequences from plasmid pR10

1. Growth of cells:

Cells were grown in a 10 liter volume in a Chemap fermentor (Chemapec, Woodbury, NY) in 2% medium. Fermentation temperature was 37°C, the pH was 6.8, and air was provided at 1 vvm. Plasmid selection was provided by 50 µg/ml ampicillin. Typical cell yield (wet weight) is 30 g/l.

2. Cell lysis:

50 g, wet cell weight, of E. coli containing the recombinant HTLV-III envelope fusion protein were

resuspended in a final volume of 100 ml in 50 mM Tris-Cl pH 8.0, 5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol (DTT), 15 mM β-mercaptoethanol, 0.5% TRITON® X-100, and 5 mM phenylmethylsulfonyl fluoride (PMSF). 300 mg lysozyme was added and the suspension incubated for 30 min at room temperature.

This material was lysed using a BEAD-BEATER™ (Biospec Products, Bartlesville, OK) containing an equal volume of 0.1-0.15 μm glass beads. The lysis was done for 6 min at room temperature in 1 min intervals. The liquid was separated from the beads and centrifuged for 2.5 hr at 20,000 xg. The supernatant was removed and the pellet dissolved in 100 ml 8 M urea, 20 mM Tris-Cl pH 8.0, 5 mM DTT, 15 mM β-mercaptoethanol, 5 mM PMSF, and 1 mM EDTA. The pellet was solubilized using a polytron homogenizer (Beckman, Berkeley, CA) and centrifuged at 20,000 xg for 2 hr.

3. Diethylaminoethyl (DEAE) chromatography:

Supernatant was loaded onto a 550 ml column (5 cm x 28 cm) packed with DEAE Fast Flow SEPHAROSE® (Pharmacia, Piscataway, NJ) equilibrated in 8 M urea, 20 mM Tris-Cl pH 8.0, 15 mM β-mercaptoethanol, and 1 mM KEDTA at room temperature. The column was washed with 1.5 liters equilibration buffer, and the protein eluted with a 5.0 liter linear gradient from 0-0.8 M NaCl in equilibration buffer. The HTLV-III protein eluted at 0.2 M NaCl and was assayed using SDS-polyacrylamide electrophoresis and following the prominent protein at approximately 95 kD.

The fractions containing the HTLV-III protein were pooled and the protein concentrated to 10 ml using a stressed cell positive pressure concentrator

(Amicon, Danvers, MA) fitted with a 10,000 MW cut-off membrane (YM-10, Amicon). The concentrate was loaded onto a 500 ml column (2.5 cm x 100 cm) packed with superfine sephacryl S-300 (Pharmacia) equilibrated in 8 M urea, 20 mM Tris-Cl, pH 8.0, 15 mM β-mercaptoethanol, and 1 mM EDTA. The column was eluted with equilibration buffer at room temperature. A flow rate of 0.5 ml/min was maintained. The HTLV-III protein eluted at approximately 40% of the column volume.

Example 4--Construction of and expression from plasmid $pPB1_{IIIB}$

Plasmid pPB1, which contains approximately 540 base pairs of DNA encoding essentially the HTLV-III *env* gene from the PvuII site to the BglII site, and from which is synthesized an approximately 26 kD fusion protein containing this portion of the gp120 envelope protein can be constructed as follows:

1. Synthesizing the DNA with the sequence shown in Table15: This DNA fragment can be synthesized by standard methods and encodes a portion of gp120. It has a blunt end on the 5' end and an end which will ligate with a BamHI overhang on the 3' end.
2. Restricting 5 µg plasmid pREV2.2 with EcoRV and BamHI and isolating the large fragment, approximately 4 kD, from an agarose gel.
3. Ligating 0.1 µg of the fragment in Table15 with 0.1 µg of the pREV2.2 fragment in a volume of 20 µl using T4 DNA ligase, transforming the ligation mixture into competent cell strain SG20251, and selecting ampicillin resistant transformants.
4. Using the AhaIII restriction pattern of purified plasmid, selecting cells harboring the recombinant

plasmid with the synthesized fragment in the orientation whereby the fragment blunt end ligated to the REV2.2 EcoRV end and the BamHI overhanging ends ligated together. AhaIII digestion of the proper plasmid gives fragment lengths of approximately 1210, 1020, 750, 690, 500, 340, and 20 base pairs. When the strain harboring this recombinant plasmid is grown in 2% medium containing 50 µg/ml ampicillin and the total complement of cellular proteins electrophoresed on an SDS-polyacrylamide gel, a protein of approximately 26 kD can be visualized by either coomassie blue staining or by western blot analysis using as probe selected sera from AIDS, ARC, or HTLV-III infected individuals.

Example 5--Purification of recombinant protein containing HTLV-III envelope sequences from plasmid pPBl$_{IIIB}$

1. Growth of cells:

Cells were grown in a 10 liter volume in a Chemap fermentor in 2% medium. Fermentation temperature was 37°C, the pH was 6.8, and air was provided at 1 vvm. Plasmid selection was provided by 50 µg/ml ampicillin and 20 µg/ml chloramphenicol. Typical cell yield (wet weight) was 30 g/l.

2. Cell Lysis:

50 g, wet cell weight, of E. coli containing the recombinant HTLV-III envelope fusion protein were resuspended in a final volume of 100 ml in 50 mM Tris-Cl pH 8.0, 5 mM EDTA, 5 mM DTT, 15 mM β-mercaptoethanol, 0.5% TRITON®X-100, and 5 mM PMSF. 300 mg lysozyme was added and the suspension incubated for 30 min at room temperature.

This material was lysed using a BEAD-BEATER™ (Biospec Products, Bartlesville, OK) containing an

equal volume of 0.1-0.15 μm glass beads. The lysis was done for 6 min at room temperature in 1 min intervals. The liquid was separated from the beads and centrifuged for 2.5 hr at 20,000 xg. The supernatant was removed and the pellet was resuspended in 100 ml 6 M guanidine-hydrochloride, 20 mM Tris-Cl pH 8.0, 5 mM DTT, 15 mM β-mercaptoethanol, 5 mM PMSF, and 1 mM EDTA. The pellet was solubilized using a polytron homogenizer and centrifuged at 20,000 xg for 2 hr.

The supernate (90 ml) was dialysed against 4 liters of 8 M urea, 20 mM potassium phosphate, pH 7.0, 1 mM EDTA, and 15 mM β-mercaptoethanol. Dialysis was done each time for 8 hr or longer with three changes of buffer. Spectraphor dialysis tubing (S/P, McGraw Park, IL) with a 3.5 kD MW cut-off was used.

3. CM chromatography

The dialysate was loaded onto a 550 ml column (5 cm x 28 cm) packed with CM Fast Flow SEPHAROSE® (Pharmacia) equilibrated in 8 M urea, 10 mM potassium phosphate pH 7.0, 15 mM β-mercaptoethanol, and 1 mM EDTA at room temperature. The column was washed with 2 liters equilibration buffer, and the protein eluted with a 5.0 liter linear gradient from 0-0.4 M NaCl. The HTLV-III protein (26 kD) eluted at approximately 0.2 M NaCl as assayed by SDS-polyacrylamide gel electrophoresis.

Example 6--Construction of and expression from plasmid p590

Plasmid p590, which contains approximately 1055 base pairs of DNA encoding essentially the HTLV-III

env gene from the PvuII site to the HindIII site, and from which is synthesized an approximately 86 kD fusion protein containing this portion of the gp160 envelope protein can be constructed as follows:

1. Synthesizing the DNA with the sequence shown in Table 6: This DNA fragment can be synthesized by standard methods and encodes a portion of gp160. It has a blunt end on the 5' end and an end which will ligate with a HindIII overhang on the 3' end.
2. Restricting 5 µg plasmid pREV2.2 with EcoRV and HindIII and isolating the large fragment, approximately 4 kD, from an agarose gel.
3. Ligating 0.1 µg of the fragment in Table 6 with 0.1 µg of the pREV2.2 fragment in a volume of 20 ml using T4 DNA ligase, transforming the ligation mixture into competent cell strain SG20251, and selecting ampicillin resistant transformants.
4. Using the AhaIII restriction pattern of purified plasmid, selectiong cells harboring the recombinant plasmid with the synthesized fragment in the orientation whereby the fragment blunt end ligated to the pREV2.2 EcoRV end and the HindIII overhanging ends ligated together. AhaIII digestion of the proper plasmid gives fragment lengths of approximately 1740, 1020, 750, 690, 500, 340, and 20.
5. 5 µg of plasmid, purified from this strain, is restricted with NdeI and SmaI. The approximately 1425 base pair fragment is isolated from an agarose gel. The 1505 base pair fragment is fused to the DNA encoding the segment of gp160.
6. 5 µg of pBG101 is restricted with BamHI, the overhanging ends filled in with Klenow polymerase and dNTPs, and then restricted with NdeI. The approximately 6.5 kD fragment is isolated from an agarose gel.

7. Ligating 0.1 µg of the NdeI-SmaI fragment with 0.1 µg of the pBG1 fragment using T4 DNA ligase, transforming the ligation mixture into competent cell strain SG20251, and selecting ampicillin resistant transformants.
8. Using the AhaIII restriction pattern of purified plasmid, selecting cells harboring the recombinant plasmid with the synthesized fragment in the orientation whereby the fragment blunt SmaI end ligated to the BamHI/filled-in end and the NdeI overhanging ends ligated together. AhaIII digestion of the proper plasmid gives fragment lengths of approximately 5900, 1020, 690, 430, and 20 base pairs.
9. When the strain harboring this recombinant plasmid is grown in 2% medium containing 50 µg/ml ampicillin and the total complement of cellular proteins electrophoresed on an SDS-polyacrylamide gel, a protein of approximately 86 kD can be visualized by either coomassie blue staining or by western blot analysis using as probe selected sera from AIDS, ARC, or HTLV-III infected individuals.

Example 7--Purification of recombinant protein containing HTLV-III envelope sequences from plasmid p590

1. Growth of cells:

Cells were grown in a 10 liter volume in a Chemap fermentor in 2% medium. Fermentation temperature was 37°C, the pH was 6.8, and air was provided at 1 vvm. Plasmid selection was provided by 50 µg/ml ampicillin. Typical cell yield (wet weight) is 30 g/l.

2. Cell Lysis:

50 g, wet cell weight, of E. coli containing the recombinant HTLV-III envelope fusion protein were

resuspended in a final volume of 100 ml in 50 mM Tris-Cl pH 8.0, 5 mM EDTA, 5 mM DTT, 15 mM β-mercaptoethanol. 0.5% TRITON®X-100, and 5 mM PMSF. 300 mg lysozyme was added and the suspension incubated for 30 min at room temperature.

This material was lysed using a Bead-Beater™ containing 0.1-0.15 mm glass beads. The lysis was done for 6 min at room temperature in 1 min intervals. The liquid was separated from the beads and centrifuged for 2.5 hr at 20,000 xg. The supernatant was removed and the pellet was resuspended in 100 ml 6 M guanidine-hydrochloride, 20 mM Tris-Cl pH 8.0, 5 mM DTT, 15 mM β-mercaptoethanol, 5 mM PMSF, and 1 mM EDTA. The pellet was solubilized using a polytron homogenizer and centrifuged at 20,000 xg for 2 hr.

The supernate (90 ml) was dialysed against 4 liters of 8 M urea, 20 mM Tris-Cl, pH 8.0, 1 mM EDTA, and 15 mM β-mercaptoethanol. Dialysis was done each time for 8 hr or longer with three changes of buffer.

3. Diethylaminoethyl (DEAE) chromatography

Dialysate was loaded onto a 550 ml column (5 cm x 28 cm) packed with DEAE Fast Flow SEPHAROSE® (Pharmacia) equilibrated in 8 M urea, 20 mM Tris-Cl pH 8.0, 15 mM β-mercaptoethanol, and 1 mM EDTA at room temperature. The column was washed with 1.5 liters equilibration buffer, and the protein eluted with a 5.0 liter linear gradient from 0-0.8 M NaCl in equilibration buffer. The HTLV-III protein eluted at 0.4 M NaCl and was assayed using SDS-polyacrylamide electrophoresis and following the prominent protein at approximately 86 kD.

The fractions containing the HTLV-III protein were pooled and the protein concentrated to 10 ml using a stressed cell positive pressure concentrator (Amicon) fitted with a 10,000 MW cut-off membrane (YM-10, Amicon). The concentrate was loaded onto a 500 ml column (2.5 cm x 100 cm) packed with superfine SEPHACRYL®S-300 (Pharmacia) equilibrated in 8 M urea, 20 mM Tris-Cl, pH 8.0, 15 mM β-mercaptoethanol, and 1 mM EDTA. The column was eluted with equilibration buffer at room temperature. A flow rate of 0.5 ml/min was maintained. The HTLV-III protein eluted at approximately 40% of the column volume.

Example 8--Construction of and expression from plasmid pKH1

Plasmid pKH1, which contains approximately 1830 base pairs of DNA encoding essentially the HTLV-III env gene from the KpnI site to the HindIII site, and from which is synthesized an approximately 70 kD fusion protein containing this portion of the gp160 envelope protein, can be constructed as follows:

1. Synthesizing the DNA with the sequence shown in Table 7: This DNA fragment can be synthesized by standard methods and encodes a portion of gp160. It has a blunt end on the 5' end and an end which will ligate with a HindIII overhang on the 3' end.
2. Restricting 5 µg plasmid pREV2.2 with MluI, treating the DNA with Klenow polymerase to blunt the ends, treating with HindIII and isolating the large fragment, approximately 5 kD, from an agarose gel.
3. Ligating 0.1 µg of the fragment in Table 7 with 0.1 µg of the pREV 2.2 fragment in a

volume of 20 µl using T4 DNA ligase, transforming the ligation mixture into competent cell strain CAG629, and selecting ampicillin resistant transformants.

4. Using the AhaIII restriction pattern of purified plasmid, selecting cells harboring the recombinant plasmid with the synthesized fragment in the orientation whereby the fragment blunt end ligated to the REV2.2 MluI end and the HindIII overhanging ends ligated together. AhaIII digestion of the proper plasmid gives fragment lengths of approximately 1730, 1020, 750, 690, 640, 600, 340, and 20 base pairs. When the strain harboring this recombinant plasmid is grown in 2% medium containing 50 µg/ml ampicillin and the total complement of cellular proteins electrophoresed on an SDS-polyacrylamide gel, a protein of approximately 70 kD can be visualized by either Coomassie blue staining or by Western Blot analysis using as probe selected sera from AIDS, ARC, or HTLV-III infected individuals.

Example 9--Purification of recombinant protein containing HTLV-III envelope sequences from plasmid pKH1

1. Growth of cells:

Cells were grown in a 10 liter volume in a Chemap fermenter in 2% medium. Fermentation temperature was 32°C, the pH was 6.8, and air was provided at 1 vvm. Plasmid selection was provided by 50 µg/ml ampicillin. Typical cell yield (wet weight) is 30 g/l.

2. Cell lysis:

50 g, wet cell weight, of E. coli containing the recombinant HTLV-III envelope fusion protein were resuspended in a final volume of 100 ml in 50 mM Tris-Cl pH 8.0, 5 mM EDTA, 5 mM dithiothreitol (DTT), 15 mM β-mercaptoethanol, 0.5% TRITON® X-100 and 5 mM PMSF. 300 mg lysozyme was added and the suspension incubated for 30 min at room temperature.

This material was lysed using a BEAD-BEATER™ (Biospec Products) containing an equal volume of 0.1-0.15 µm glass beads. The lysis was done for 6 min at room temperature in 1 min intervals. The liquid was separated from the beads and centrifuged for 2.5 hr at 20,000 xg. The supernatant was removed and the pellet dissolved in 100 ml 8 M urea, 20 mM Tris-Cl pH 8.0, 5 mM DTT, 15 mM β-mercaptoethanol, 5 mM PMSF, and 1 mM EDTA. The pellet was solubilized using a polytron homogenizer (Beckman, Berkeley, CA) and centrifuged at 20,000 xg for 2 hr.

3. DEAE chromatography:

Supernatant was loaded onto a 550 ml column (5 cm x 28 cm) packed with DEAE Fast Flow SEPHAROSE® (Pharmacia) equilibrated in 8 M urea, 20 mM Tris-Cl pH 8.0, 15 mM β-mercaptoethanol, and 1 mM EDTA at room temperature. The column was washed with 1.5 liters equilibration buffer, and the protein eluted with a 5.0 liter linear gradient from 0-0.8 M NaCl in equilibration buffer. The HTLV-III protein eluted at 0.2 M NaCl and was assayed using SDS-polyacrylamide electrophoresis and following the protein at approximately 70 kD.

The fractions containing the HTLV-III protein were pooled and the protein concentrated to 10 ml using a stressed cell positive pressure concentrator

(Amicon) fitted with a 10,000 MW cut-off membrane (YM-10, Amicon). The concentrate was loaded onto a 500 ml column (2.5 cm x 100 cm) packed with superfine SEPHACRYL® S-300 (Pharmacia) equilibrated in 8 M urea, 20 mM Tris-Cl, pH 8.0, 15 mM β-mercaptoethanol, and 1 mM EDTA. The column was eluted with equilibration buffer at room temperature. A flow rate of 0.5 ml/min was maintained. The HTLV-III protein eluted at approximately 40% of the column volume.

4. SDS-polyacrylamide electrophoresis:

The fractions containing KH1 were pooled and the protein concentrated using a stressed cell positive pressure concentrator fitted with a 10,000 MW cutoff membrane. 2 mg of protein was mixed with loading buffers and electrophoresed through a preparative SDS-polyacrylamide gel (40 cm x 20 cm x 4 mm) as described by M.W. Humkapiller, E. Lujan, F. Ostrander, and L.E. Hood, Methods in Enzymology 91:227-236 (1983). The 70 kD HTLV-III protein was visualized with 0.25 M KCl and eluted from the gel as described. The protein can be removed from the SDS by precipitation with acetone (Dynan, W.J., Jendrisak, J.J., Hager, D.A. and Burgess, R.R. [1981] J. Biol. Chem. 256:5860-5865).

Example 10--Construction of a non-fusion derivative of PB1

A non-fusion derivative of the PB1 protein containing no non-HTLV-III amino acids other than an N-terminal methionine was constructed using oligonucleotide-directed site-specific mutagenesis (Inouye, S. and Inouye, M.,

"Synthesis & Applications of DNA & RNA", ed. Narang, Saran A. Academic Press, 1987). In this procedure, 90 non-HTLV-III bp upstream and 39 downstream of the env gene sequence in pPB1 were deleted via DNA loopouts generated by hybridization with synthetic oligonucleotides.

The oligonucleotide synthesized for the N-terminal loopout was designed so that the start codon of the β-glucuronidase gene is placed immediately adjacent to the 5' end of the HTLV-III env gene sequence (Figure 4). The oligonucleotide includes sequences homologous to both sides of this newly-created junction that allow proper hybridization to the plasmid DNA.

The two DNA molecules used to form a heteroduplex with a single-stranded gap that is the substrate for hybridization were created by digesting pPB1 with SalI and HpaI, or with PstI alone. Digestion with PstI linearized pPB1, and a double digest with SalI and HpaI yields fragments of 3800 and 700 bp, the larger of which was gel-isolated for use in the mutagenesis.

Kinasing of the oligonucleotide, hybridization, polymerization and ligation to yield closed circular molecules were done according to the methods of Inouye and Inouye mentioned above. To enrich for DNA molecules containing the deletion, the DNA mixture was digested with MluI, which cuts within the region being deleted.

The digested DNA was used to transform competent E. coli JM105 cells and plasmid-containing transformants were isolated by overnight growth on YT (8 g tryptone, 5 g yeast extract, and 5 g NaCl per liter) Cm plates at 37°C.

Plasmid DNA was isolated from each transformant and screened for the correct construction by simultaneous digestion with MluI and HindIII. Molecules that were not deleted yielded fragments of approximately 3900 and 600 bp. Those containing the deletion did not have the MluI site and yielded linear molecules of approximately 4400 bp. Plasmid DNA from transformants that appeared to contain the deletion was retransformed to ensure segregation of deleted and non-deleted plasmids and the recovery of pure plasmid populations. DNA from these second transformants was analyzed as in the previous digest and was determined to have the correct construction. This plasmid was designated pΔPB1.

To eliminate the C-terminal non-HTLV-III amino acids, oligonucleotide-directed site-specific mutagenesis was carried out as above, using the pΔPB1 plasmid as a substrate. The oligonucleotide (Figure 5) was designed to position the TGA codon that occurs out-of-frame downstream from the env gene sequence so that it is immediately adjacent to the 3' end of the env gene sequence and in-frame to act as a translational stop codon.

The molecules to form the heteroduplex used for hybridization were created by digesting pΔPB1 with PstI alone or with KpnI and HpaI. The large KpnI/HpaI fragment encompassing most of the vector was gel-isolated for use in the mutagenesis. Kinasing, hybridization, polymerization and ligation were performed as above. Enrichment for deleted molecules was accomplished by digesting with HindIII, which cuts within the region being deleted. The DNA was used to transform cells as above.

Plasmid DNA was isolated from each transformant and screened for the correct construction by simultaneous digestion with EcoRI and HpaI. The deleted plasmid yields two restriction fragments of 2900 and 1750 bp. Plasmid DNA showing this pattern was retransformed as above, and DNA from these transformants was analyzed with the same digest. This plasmid, containing N-terminal and C-terminal deletions, is designated pd2PB1.

When the strain harboring plasmid pΔPB1 is grown in 2% medium (2% yeast extract, bactotryptone, casamino acids [Difco, Detroit, MI], 0.2% potassium monobasic, 0.2% potassium dibasic, and 0.2% sodium dibasic) containing 50 µg/ml ampicillin and the total complement of cellular proteins electrophoresed on an SDS-polyacrylamide gel, a protein of approximately 22 kD can be visualized by either coomassie blue staining or by western blot analysis using as probe selected sera from animals immunized with recombinant env gene proteins. Under the same conditions, a protein of approximately 20 kD is produced in a strain containing pd2PB1.

The technique of oligonucleotide-directed site-specific mutagenesis can be used in a similar way to eliminate the non-HTLV-III amino acids flanking the env gene fusion proteins R10, 590, and KH1.

In the procedure detailed above, the removal of the non-HTLV-III sequences from the fusion proteins involves removal of amino acids at both the N-terminus and the C-terminus of the protein and is accomplished in two sequential steps.

It is well known in the art that a methionine at the N-terminal position can be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP).

MAP has been cloned from E. coli (Ben-Bassat, A., Bauer, K., Chang, S.-Y., Myambo, K., Boosman, A. and Chang, S. [1987] Journal of Bacteriology 169(2):751-757) and Salmonella typhimurium, and in vitro activity has been demonstrated on recombinant proteins (Miller, C.G., Strauch, K.L., Kukral, A.M., Miller, J.L., Wingfield, P.T., Massei, G.J., Werlen, R.C., Graber, P. and Movva, N.R. [1987] Proc. Natl. Acad. Sci. USA 84:2718-2722). Therefore, removal of an N-terminal methionine may be achieved either in vivo by expressing the protein in a host which produces MAP (e.g., E. coli CM89 or S. cerevisiae), or in vitro by use of purified MAP (e.g., procedure of Miller et al.).

pd2PB1 Purification

Unless specified otherwise, all steps are carried out at room temperature.

Lysis--Three 700 ml bottles of frozen cell paste containing pd2PB1 are thawed at 37°C, and are then spun at 4,000 rpm in a J-6B centrifuge with a JS-4.2 rotor (Beckman, Palo Alto, CA) at 4°C for 30 min. The supernatant is then discarded and the weight of the cell pellet is determined. The cell pellet (typically 1 kg) is resuspended in 2 volumes of lysis buffer (v/w) which consists of 8 M urea, 20 mM Tris-HCl (pH 7.5 ± 0.1), 1 mM EDTA, 14.7 mM 2-mercaptoethanol and 1 mM PMSF.

The resuspended cell pellet is run through a Type TDK Pilot DYNO-MILL® (Impandex Inc., Maywood, NJ) containing 0.5-0.7 mm glass beads at 200-400 ml/min. Prior to use the DYNO-MILL® is charged with one liter of lysis buffer and cooled so that the solution flowing through is at less than ambient temperature. The resuspended cell pellet is passed through the DYNO-MILL® twice,

and after the second pass, the DYNO-MILL® is washed with 1 liter of lysis buffer. Lysed cell suspension and wash are pooled.

Concentration and filtration--The lysed cell suspension plus one liter wash is concentrated to 800 ml using a 0.45 micron DURAPORE™ Pellicon cassette in a Pellicon 4 GPM system (Millipore, Bedford, MA). The concentration is done with an inlet pressure of less than or equal to 40 psi and an outlet pressure between 10 and 20 psi. After concentration the lysed cell suspension is filtered with 4 liters of lysis buffer using the same Pellicon system, cassette and pressure settings with the tubing rigged for dyafiltration.

Extraction--The washed lysis cell suspension is extracted with 10 l of extraction buffer consisting of 6 M guanidine HCl, 100 mM Tris-HCl (pH 7.6 ± 0.1), and 10 mM EDTA, using the same Pellicon system, cassette and pressure settings as described above with the tubing rigged for dyafiltration.

Buffer exchange--The filtrate from the previous step is typically concentrated to 1 liter using a Pellicon 4GPM system with two PTGC cassettes (10,000 NMWL). The concentration is done with an inlet pressure of less than or equal to 50 psi and an outlet pressure between 30 and 45 psi. After concentration, the supernatant is buffer exchanged with CM column buffer consisting of 8 M urea, 25 mM potassium phosphate, and 1 mM EDTA (pH 6.8 ± 0.1), with conductivity less than or equal to 3.0 ms/cm. For buffer exchange, the same Pellicon system, the same cassettes and the same pressure settings as above are used with the tubing rigged for dyafiltration. Eight liters of CM column buffer are used to buffer exchange 1 liter of concentrated extract. After buffer exchange,

the buffer-exchanged extract is drained from the system and the system is washed with 1 liter of CM column buffer. The buffer-exchanged extract and the wash are pooled and the solution's conductivity and pH are measured. The conductivity of the solution is adjusted to less than or equal to 3.0ms/cm with deionized 8 M urea and the pH is adjusted to be within the range of 6.5-7.0.

CM chromatography--A 50 x 51 cm column of CM SEPHAROSE® FAST FLOW (Pharmacia, Piscataway, NJ) is equilibrated by washing the column sequentially with 4 column volumes of 0.5 M NaOH, 2 column volumes of deionized water and 2-3 column volumes of CM column buffer. The column is considered equilibrated when the pH of the outflow is within 0.2 units of the CM column buffer and the conductivity of the outflow is within 0.3 ms/cm of the CM column buffer.

For loading, the buffer exchanged extract is pumped on to the column at an inlet pressure between 10 and 15 psi. After loading, the CM column is washed with CM column buffer until the OD at 280 nm of the outflow is less than 0.1. The pd2PB1 is then eluted with an 8-liter linear gradient of 0-0.5 M NaCl in CM column buffer and collected in 100 ml fractions. The fractions are assayed by SDS-PAGE and Western with anti-gp160 antibody, and those containing significant pd2PB1 and trace contaminants are pooled.

Organic extraction--The pooled protein solution from the previous step is brought to a ratio of 55% acetonitrile to 45% protein solution (v/v) by the slow addition of pure acetonitrile with mixing. After addition of all of the acetonitrile, the solution is centrifuged in a J2-21 centrifuge using a JA10 rotor (Beckman) at 10,000 rpm and 4°C for 15 min. After centrifugation, the supernatant is collected and the pellet is discarded.

The centrifugation supernatant is brought to a ratio of 35% ethanol to 65% supernatant (v/v) by slow addition of 95% ethanol with mixing. After addition of all of the ethanol, the solution is centrifuged in a J2-21 centrifuge using a JA-10 rotor at 10,000 rpm and 4°C for 15 min. After centrifugation the pellet is collected and the supernatant is discarded.

The pellet is allowed to air dry for 15 min, and is then redissolved in S-300 column buffer, which consists of 8 M urea, 0.3 M glycine, 5 mM EDTA, 15 mM 2-mercapto-ethanol, 1 mM dithiothreitol (DTT) (pH 8.50 ± 0.01). The pellet is dissolved in a volume of S-300 column buffer equal to one-tenth the volume of the pooled protein solution at the beginning of this step.

Concentration--The absorbance of the redissolved protein solution from above is determined at 280 nm and an approximate protein concentration is determined by assuming that a 1 mg/ml solution of protein has an absorbance of 1.0 at 280 nm. The solution is concentrated to 10 mg/ml using a 200 ml Amicon stirred cell concentrator with a YM-10 membrane.

S-300 chromatography--Thirty to seventy ml of the concentrated protein solution is loaded on a 5.0 x 135 cm column of SEPHACRYL® S-300 from Pharmacia. The column had been previously equilibrated with S-300 column buffer which consists of 8 M urea, 0.3 M glycine, 5 mM EDTA, 15 mM 2-mercaptoethanol, 1 mM DTT (pH 8.50 ± 0.01). After loading, the column is run isocratically in the same buffer. Twenty ml fractions are collected and the fractions are assayed for pd2PB1 content by SDS-PAGE.

Equal volume aliquots are taken from suitable fractions containing pd2PB1 and are used to determine which fractions are satisfactory for pooling. The aliquots are pooled, dialyzed overnight versus 8 M urea, 25 mM sodium phosphate, 1 mM EDTA (pH 6.8 ± 0.1), and the OD at 280 nm of the dialyzed pool is determined using the dialysis buffer as blank. The protein concentration of the solution is determined using the calculated extinction coefficient of pd2PB1 of 1.0 $(mg/ml)^{-1}$. SDS-PAGE is run on 10 µg of the dialyzed pooling using a 15% SDS acrylamide gel. After coomassie staining and destaining, the gel is scanned using an LKB (Gaithersburg, MD) scanning densitometer attached to a Waters (Milford, MA) 740 Integrator. If the pd2PB1 band on the gel is more than 97% pure, then the fractions that were used for the aliquot are checked for endotoxins at a 1 to 20 dilution in the Limulus Amebocyte Lysate (LAL) assay using 0.06 eu/ml tubes. If the LAL test on the diluted fractions is negative, the fractions are pooled and used for subsequent operations. If the gel fails to meet the purity specification, the process is repeated using equal volume aliquots from a different set of fractions. Only those fractions having a negative LAL test at a 1 to 20 dilution are pooled.

Table 1

```
5' GATCAAGCTTCTGCAGTCGACGCATGCGGATCCGGTACCCGGGAGCTCG 3'
    TTCGAAGACGTCAGCTGCGTACGCCTAGGCCATGGGCCCTCGAGCTTAA
```

Table 2

```
5'     CGGTACCAGCCCGCCTAATGAGCGGGCTTTTTTTTGACGT    3'
   TGCAGCCATGGTCGGGCGGATTACTCGCCCGAAAAAAAAC
```

Table 3

```
    MluI      EcoRV  ClaI BamHI  SalI HindIII SmaI

CGAACGCGTGGCCGATATCATCGATGGATCCGTCGACAAGCTTCCCGGGAGCT
GCTTGCGCACCGGCTATAGTAGCTACCTAGGCAGCTGTTCGAAGGGCCC
```

Table 4

```
                5'   AATTCCCTGTGTGGAAGGAAGCA
                     TTAAGGGACACACCTTCCTTCGT

ACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAGAGGTACAT
TGGTGGTGAGATAAAACACGTAGTCTACGATTTCGTATACTATGTCTCCATGTA

AATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTA
TTACAAACCCGGTGTGTACGGACACATGGGTGTCTGGGGTTGGGTGTTCTTCAT

GTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAA
CATAACCATTTACACTGTCTTTTAAAATTGTACACCTTTTTACTGTACCATCTT

CAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTA
GTCTACGTACTCCTATATTAGTCAAATACCCTAGTTTCGGATTTCGGTACACAT

AAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGATTTGAAGAATGATACT
TTTAATTGGGGTGAGACACAATCAAATTTCACGTGACTAAACTTCTTACTATGA

AATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAAAC
TTATGGTTATCATCATCGCCCTCTTACTATTACCTCTTTCCTCTCTATTTTTTG

TGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAGAAAGAATATGCA
ACGAGAAAGTTATAGTCGTGTTCGTATTCTCCATTCCACGTCTTTCTTATACGT

TTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
AAAAAAATATTTGAACTATATTATGGTTATCTATTACTATGATGGTCGATATGC

TTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTT
AACTGTTCAACATTGTGGAGTCAGTAATGTGTCCGGACAGGTTTCCATAGGAAA

GAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGT
CTCGGTTAAGGGTATGTAATAACACGGGGCCGACCAAAACGCTAAGATTTTACA

AATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTCAGCACAGTACAA
TTATTATTCTGCAAGTTACCTTGTCCTGGTACATGTTTACAGTCGTGTCATGTT

TGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAATGGCAGT
ACATGTGTACCTTAATCCGGTCATCATAGTTGAGTTGACGACAATTTACCGTCA

CTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAA
GATCGTCTTCTTCTCCATCATTAATCTAGACGGTTAAAGTGTCTGTTACGATTT

ACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAAC
TGGTATTATCATGTCGACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTG

AACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTT
TTGTTATGTTCTTTTTCATAGGCATAGGTCTCTCCTAATCCCTCTCGTAAACAA
```

Table 4 (cont.)

```
ACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
TGTTATCCTTTTTATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGT

AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGA
TTTACCTTATTGTGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCT

AATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTA
TTATTATTTTGTTATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACAT

ACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTG
TGCGTGTCAAAATTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGAC

TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACT
AAATTATCATGAACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGA

GAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATG
CTTCCTTCACTGTGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTAC

TGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGA
ACCGTCCTTCATCCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCT

TGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAAC
ACAAGTAGTTTATAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTG

AATGAGTCCGA          3'
TTACTCAGGCTCTAG
```

Table 5

```
                5'  CTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAAC
                    GACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTG

AACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTT
TTGTTATGTTCTTTTTCATAGGCATAGGTCTCTCCTGGTCCCTCTCGTAAACAA

ACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
TGTTATCCTTTTTATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGT

AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGA
TTTACCTTATTGTGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCT

AATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTA
TTATTATTTTGTTATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACAT

ACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTG
TGCGTGTCAAAATTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGAC

TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACT
AAATTATCATGAACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGA

GAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATG
CTTCCTTCACTGTGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTAC

TGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGA
ACCGTCCTTCATCCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCT

TGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAAC
ACAAGTAGTTTATAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTG

AATGAGTCCGA          3'
TTACTCAGGCTCTAG
```

Table 6

```
5'  CTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAAC
    GACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTG

AACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTT
TTGTTATGTTCTTTTTCATAGGCATAGGTCTCTCCTGGTCCCTCTCGTAAACAA

ACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
TGTTATCCTTTTTATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGT

AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGA
TTTACCTTATTGTGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCT

AATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTA
TTATTATTTTGTTATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACAT

ACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTG
TGCGTGTCAAAATTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGAC

TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACT
AAATTATCATGAACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGA

GAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATG
CTTCCTTCACTGTGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTAC

TGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGA
ACCGTCCTTCATCCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCT

TGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAAC
ACAAGTAGTTTATAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTG

AATGAGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGA
TTACTCAGGCTCTAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTTAACCTCT

AGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCC
TCACTTAATATATTTATATTTCATCATTTTTAACTTGGTAATCCTCATCGTGGG

ACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGA
TGGTTCCGTTTCTCTTCTCACCACGTCTCTCTTTTTTCTCGTCACCCTTATCCT

GCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCA
CGAAACAAGGAACCCAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGT

ATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAG
TACTGCGACTGCCATGTCCGGTCTGTTAATAACAGACCATATCACGTCGTCGTC

AACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTC
TTGTTAAACGACTCCCGATAACTCCGCGTTGTCGTAGACAACGTTGAGTGTCAG

TGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAG
ACCCCGTAGTTCGTCGAGGTCCGTTCTTAGGACCGACACCTTTCTATGGATTTC
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Table 6 (cont.)

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GATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACT
CTAGTTGTCGAGGACCCCTAAACCCCAACGAGACCTTTTGAGTAAACGTGGTGA

GCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAAT
CGACACGGAACCTTACGATCAACCTCATTATTTAGAGACCTTGTCTAAACCTTA

AACATGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACA        3'
TTGTACTGGACCTACCTCACCCTGTCTCTTTAATTGTTAATGTGTTCGA
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Table 7

```
5'    AATTCCCTGTGTGGAAGGAAGCA
      TTAAGGGACACACCTTCCTTCGT

ACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAGAGGTACAT
TGGTGGTGAGATAAAACACGTAGTCTACGATTTCGTATACTATGTCTCCATGTA

AATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTA
TTACAAACCCGGTGTGTACGGACACATGGGTGTCTGGGGTTGGGTGTTCTTCAT

GTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAA
CATAACCATTTACACTGTCTTTTAAAATTGTACACCTTTTTACTGTACCATCTT

CAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTA
GTCTACGTACTCCTATATTAGTCAAATACCCTAGTTTCGGATTTCGGTACACAT

AAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGATTTGAAGAATGATACT
TTTAATTGGGGTGAGACACAATCAAATTTCACGTGACTAAACTTCTTACTATGA

AATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAAAC
TTATGGTTATCATCATCGCCCTCTTACTATTACCTCTTTCCTCTCTATTTTTG

TGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAGAAAGAATATGCA
ACGAGAAAGTTATAGTCGTGTTCGTATTCTCCATTCCACGTCTTTCTTATACGT

TTTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
AAAAAATATTTGAACTATATTATGGTTATCTATTACTATGATGGTCGATATGC

TTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTT
AACTGTTCAACATTGTGGAGTCAGTAATGTGTCCGGACAGGTTTCCATAGGAAA

GAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGT
CTCGGTTAAGGGTATGTAATAACACGGGGCCGACCAAAACGCTAAGATTTTACA

AATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTCAGCACAGTACAA
TTATTATTCTGCAAGTTACCTTGTCCTGGTACATGTTTACAGTCGTGTCATGTT

TGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAATGGCAGT
ACATGTGTACCTTAATCCGGTCATCATAGTTGAGTTGACGACAATTTACCGTCA

CTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAA
GATCGTCTTCTTCTCCATCATTAATCTAGACGGTTAAAGTGTCTGTTACGATTT

ACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAAC
TGGTATTATCATGTCGACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTG

AACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTT
TTGTTATGTTCTTTTTCATAGGCATAGGTCTCTCCTAATCCCTCTCGTAAACAA

ACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
TGTTATCCTTTTTATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGT
```

Table 7 (cont.)

```
AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGA
TTTACCTTATTGTGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCT

AATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTA
TTATTATTTTGTTATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACAT

ACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTG
TGCGTGTCAAAATTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGAC

TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACT
AAATTATCATGAACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGA

GAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATG
CTTCCTTCACTGTGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTAC

TGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGA
ACCGTCCTTCATCCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCT

TGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAAC
ACAAGTAGTTTATAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTG

AATGAGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGA
TTACTCAGGCTCTAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTTAACCTCT

AGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCC
TCACTTAATATATTTATATTTCATCATTTTTAACTTGGTAATCCTCATCGTGGG

ACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGA
TGGTTCCGTTTCTCTTCTCACCACGTCTCTCTTTTTTCTCGTCACCCTTATCCT

GCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCA
CGAAACAAGGAACCCAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGT

ATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAG
TACTGCGACTGCCATGTCCGGTCTGTTAATAACAGACCATATCACGTCGTCGTC

AACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTC
TTGTTAAACGACTCCCGATAACTCCGCGTTGTCGTAGACAACGTTGAGTGTCAG

TGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAG
ACCCCGTAGTTCGTCGAGGTCCGTTCTTAGGACCGACACCTTTCTATGGATTTC

GATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACT
CTAGTTGTCGAGGACCCCTAAACCCCAACGAGACCTTTTGAGTAAACGTGGTGA

GCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAAT
CGACACGGAACCTTACGATCAACCTCATTATTTAGAGACCTTGTCTAAACCTTA

AACATGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACA          3'
TTGTACTGGACCTACCTCACCCTGTCTCTTTAATTGTTAATGTGTTCGA
```

Table 8

Amino acid sequence of fusion protein R10

MetLeuArg
ProValGluThrProThrArgGluIleLysLysLeuAspGlyLeuTrpAlaPhe
SerLeuAspArgGluAsnCysGlyIleAspGlnPheProValTrpLysGluAla
ThrThrThrLeuPheCysAlaSerAspAlaLysAlaTyrAspThrGluValHis
AsnValTrpAlaThrHisAlaCysValProThrAspProAsnProGlnGluVal
ValLeuValAsnValThrGluAsnPheAsnMetTrpLysAsnAspMetValGlu
GlnMetHisGluAspIleIleSerLeuTrpAspGlnSerLeuLysProCysVal
LysLeuThrProLeuCysValSerLeuLysCysThrAspLeuLysAsnAspThr
AsnThrAsnSerSerSerGlyArgMetIleMetGluLysGlyGluIleLysAsn
CysSerPheAsnIleSerThrSerIleArgGlyLysValGlnLysGluTyrAla
PhePheTyrLysLeuAspIleIleProIleAspAsnAspThrThrSerTyrThr
LeuThrSerCysAsnThrSerValIleThrGlnAlaCysProLysValSerPhe
GluProIleProIleHisTyrCysAlaProAlaGlyPheAlaIleLeuLysCys
AsnAsnLysThrPheAsnGlyThrGlyProCysThrAsnValSerThrValGln
CysThrHisGlyIleArgProValValSerThrGlnLeuLeuLeuAsnGlySer
LeuAlaGluGluGluValValIleArgSerAlaAsnPheThrAspAsnAlaLys
ThrIleIleValGlnLeuAsnGlnSerValGluIleAsnCysThrArgProAsn
AsnAsnThrArgLysSerIleArgIleGlnArgGlyProGlyArgAlaPheVal
ThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCysAsnIleSerArgAla
LysTrpAsnAsnThrLeuLysGlnIleAspSerLysLeuArgGluGlnPheGly
AsnAsnLysThrIleIlePheLysGlnSerSerGlyGlyAspProGluIleVal
ThrHisSerPheAsnCysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeu
PheAsnSerThrTrpPheAsnSerThrTrpSerThrLysGlySerAsnAsnThr
GluGlySerAspThrIleThrLeuProCysArgIleLysGlnIleIleAsnMet
TrpGlnGluValGlyLysAlaMetTyrAlaProProIleSerGlyGlnIleArg
CysSerSerAsnIleThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsn

Table 8 (cont.)

AsnGluSerGluIleHisArgSerValMetLeuTyrThrThrProAsnThrTrp
ValAspAspIleThrValValThrHisValAlaGlnAspCysAsnHisAlaSer
ValAspTrpGlnValValAlaAsnGlyAspValSerValGluLeuArgAspAla
AspGlnGlnValValAlaThrGlyGlnGlyThrSerGlyThrLeuGlnValVal
AsnProHisLeuTrpGlnProGlyGluGlyTyrLeuTyrGluLeuCysValThr
AlaLysSerGlnThrGluCysAspIleTyrProLeuArgValGlyIleArgSer
ValAlaValLysGlyGluGlnPheLeuIleAsnHisLysProPheTyrPheThr
GlyPheGlyArgHisGluAspAlaAspLeuArgGlyLysGlyPheAspAsnVal
LeuMetValHisAspHisAlaLeuMetAspTrpIleGlyAlaAsnSerTyrArg
ThrSerHisTyrProTyrAlaGluGluMetLeuAspTrpAlaAspGluHisGly
IleValValIleAspGluThrAlaAlaValGlyPheAsnLeuSerLeuGlyIle
GlyPheGluAlaGlyAsnLysProLysGluLeuTyrSerGluGluAlaValAsn
GlyGluThrGlnGlnAlaHisLeuGlnAlaIleLysGluLeuIleAlaArgAsp
LysAsnHisProSerValValMetTrpSerIleAlaAsnGluProAspThrArg
ProGlnGlyAlaArgGluTyrPheAlaProLeuAlaGluAlaThrArgLysLeu
AspProThrArgProIleThrCysValAsnValMetPheCysAspAlaHisThr
AspThrIleSerAspLeuPheAspValLeuCysLeuAsnArgTyrTyrGlyTrp
TyrValGlnSerGlyAspLeuGluThrAlaGluLysValLeuGluLysGluLeu
LeuAlaTrpGlnGluLysLeuHisGlnProIleIleIleThrGluTyrGlyVal
AspThrLeuAlaGlyLeuHisSerMetTyrThrAspMetTrpSerGluGluTyr
GlnCysAlaTrpLeuAspMetTyrHisArgValPheAspArgValSerAlaVal
ValGlyGluGlnValTrpAsnPheAlaAspPheAlaThrSerGlnGlyIleLeu
ArgValGlyGlyAsnLysLysGlyIlePheThrArgAspArgLysProLysSer
AlaAlaPheLeuLeuGlnLysArgTrpThrGlyMetAsnPheGlyGluLysPro
GlnGlnGlyGlyLysGln

Table 8A

Nucleotide sequence encoding fusion protein R10

```
                                                    ATGTTACGT
                                                    TACAATGCA

CCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTGTGGGCATTC
GGACATCTTTGGGGTTGGGCACTTTAGTTTTTTGAGCTGCCGGACACCCGTAAG

AGTCTGGATCGCGAAAACTGTGGAATTGATCAATTCCCTGTGTGGAAGGAAGCA
TCAGACCTAGCGCTTTTGACACCTTAACTAGTTAAGGGACACACCTTCCTTCGT

ACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAGAGGTACAT
TGGTGGTGAGATAAAACACGTAGTCTACGATTTCGTATACTATGTCTCCATGTA

AATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTA
TTACAAACCCGGTGTGTACGGACACATGGGTGTCTGGGGTTGGGTGTTCTTCAT

GTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAA
CATAACCATTTACACTGTCTTTTAAAATTGTACACCTTTTTACTGTACCATCTT

CAGATGCATGAGGATATAAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTA
GTCTACGTACTCCTATATTAGTCAAATACCCTAGTTTCGGATTTCGGTACACAT

AAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGATTTGAAGAATGATACT
TTTAATTGGGGTGAGACACAATCAAATTTCACGTGACTAAACTTCTTACTATGA

AATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAAAC
TTATGGTTATCATCATCGCCCTCTTACTATTACCTCTTTCCTCTCTATTTTTG

TGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTGCAGAAAGAATATGCA
ACGAGAAAGTTATAGTCGTGTTCGTATTCTCCATTCGACGTCTTTCTTATACGT

TTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
AAAAAAATATTTGAACTATATTATGGTTATCTATTACTATGATGGTCGATATGC

TTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGTCCGAAGGTATCCTTT
AACTGTTCAACATTGTGGAGTCAGTAATGTGTCCGGACAGGTTTCCATAGGAAA

GAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTTGCGATTCTAAAATGT
CTCGGTTAAGGGTATGTAATAACACGGGGCCGACCAAAACGCTAAGATTTTACA

AATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTCAGCACAGTACAA
TTATTATTCTGCAAGTTACCTTGTCCTGGTACATGTTTACAGTCGTGTCATGTT

TGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAATGGCAGT
ACATGTGTACCTTAATCCGGTCATCATAGTTGAGTTGACGACAATTTACCGTCA

CTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAA
GATCGTCTTCTTCTCCATCATTAATCTAGACGGTTAAAGTGTCTGTTACGATTT

ACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAAC
TGGTATTATCATGTCGACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTG
```

Table 8A (cont.)

AACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTT
TTGTTATGTTCTTTTTCATAGGCATAGGTCTCTCCTAATCCCTCTCGTAAACAA

ACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
TGTTATCCTTTTTATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGT

AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGA
TTTACCTTATTGTGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCT

AATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTA
TTATTATTTTGTTATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACAT

ACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTG
TGCGTGTCAAAATTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGAC

TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACT
AAATTATCATGAACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGA

GAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATG
CTTCCTTCACTGTGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTAC

TGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGA
ACCGTCCTTCATCCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCT

TGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAAC
ACAAGTAGTTTATAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTG

AATGAGTCCGAGATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGG
TTACTCAGGCTCTAGGTAGCGTCGCATTACGAGATGTGGTGCGGCTTGTGGACC

GTGGACGATATCACCGTGGTGACGCATGTCGCGCAAGACTGTAACCACGCGTCT
CACCTGCTATAGTGGCACCACTGCGTACAGCGCGTTCTGACATTGGTGCGCAGA

GTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCG
CAACTGACCGTCCACCACCGGTTACCACTACAGTCGCAACTTGACGCACTACGC

GATCAACAGGTGGTTGCAACTGGACAAGGCACTAGCGGGACTTTGCAAGTGGTG
CTAGTTGTCCACCAACGTTGACCTGTTCCGTGATCGCCCTGAAACGTTCACCAC

AATCCGCACCTCTGGCAACCGGGTGAAGGTTATCTCTATGAACTGTGCGTCACA
TTAGGCGTGGAGACCGTTGGCCCACTTCCAATAGAGATACTTGACACGCAGTGT

GCCAAAAGCCAGACAGAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCA
CGGTTTTCGGTCTGTCTCACACTATAGATGGGCGAAGCGCAGCCGTAGGCCAGT

GTGGCAGTGAAGGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACT
CACCGTCACTTCCCGCTTGTCAAGGACTAATTGGTGTTTGGCAAGATGAAATGA

GGCTTTGGTCGTCATGAAGATGCGGACTTGCGTGGCAAAGGATTCGATAACGTG
CCGAAACCAGCAGCACTTCTACGCCTGAACGCACCGTTTCCTAAGCTATTGCAC

CTGATGGTGCACGACCACGCATTAATGGACTGGATTGGGGCCAACTCCTACCGT
GACTACCACGTGCTGGTGCGTAATTACCTGACCTAACCCCGGTTGAGGATGGCA

Table 8A (cont.)

ACCTCGCATTACCCTTACGCTGAAGAGATGCTCGACTGGGCAGATGAACATGGC
TGGAGCGTAATGGGAATGCGACTTCTCTACGAGCTGACCCGTCTACTTGTACCG

ATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTAACCTCTCTTTAGGCATT
TAGCACCACTAACTACTTTGACGACGACAGCCGAAATTGGAGAGAAATCCGTAA

GGTTTCGAAGCGGGCAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAAC
CCAAAGCTTCGCCCGTTGTTCGGCTTTCTTGACATGTCGCTTCTCCGTCAGTTG

GGGGAAACTCAGCAAGCGCACTTACAGGCGATTAAAGAGCTGATAGCGCGTGAC
CCCCTTTGAGTCGTTCGCGTGAATGTCCGCTAATTTCTCGACTATCGCGCACTG

AAAAACCACCCAAGCGTGGTGATGTGGAGTATTGCCAACGAACCGGATACCCGT
TTTTTGGTGGGTTCGCACCACTACACCTCATAACGGTTGCTTGGCCTATGGGCA

CCGCAAGGTGCACGGGAATATTTCGCGCCACTGGCGGAAGCAACGCGTAAACTC
GGCGTTCCACGTGCCCTTATAAAGCGCGGTGACCGCCTTCGTTGCGCATTTGAG

GACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCGACGCTCACACC
CTGGGCTGCGCAGGCTAGTGGACGCAGTTACATTACAAGACGCTGCGAGTGTGG

GATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGGATGG
CTATGGTAGTCGCTAGAGAAACTACACGACACGGACTTGGCAATAATGCCTACC

TATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGAACTT
ATACAGGTTTCGCCGCTAAACCTTTGCCGTCTCTTCCATGACCTTTTTCTTGAA

CTGGCCTGGCAGGAGAAACTGCATCAGCCGATTATCATCACCGAATACGGCGTG
GACCGGACCGTCCTCTTTGACGTAGTCGGCTAATAGTAGTGGCTTATGCCGCAC

GATACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTAT
CTATGCAATCGGCCCGACGTGAGTTACATGTGGCTGTACACCTCACTTCTCATA

CAGTGTGCATGGCTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTC
GTCACACGTACCGACCTATACATAGTGGCGCAGAAACTAGCGCAGTCGCGGCAG

GTCGGTGAACAGGTATGGAATTTCGCCGATTTTGCGACCTCGCAAGGCATATTG
CAGCCACTTGTCCATACCTTAAAGCGGCTAAAACGCTGGAGCGTTCCGTATAAC

CGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCG
GCGCAACCGCCATTGTTCTTTCCCTAGAAGTGAGCGCTGGCGTTTGGCTTCAGC

GCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCG
CGCCGAAAAGACGACGTTTTTGCGACCTGACCGTACTTGAAGCCACTTTTTGGC

CAGCAGGGAGGCAAACAA
GTCGTCCCTCCGTTTGTT

Table 9
Amino acid sequence of fusion protein PB1

MetLeuArg
ProValGluThrProThrArgGluIleLysLysLeuAspGlyLeuTrpAlaPhe
SerLeuAspArgGluArgValAlaAspLeuAsnGlnSerValGluIleAsnCys
ThrArgProAsnAsnAsnThrArgLysSerIleArgIleGlnArgGlyProGly
ArgAlaPheValThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCysAsn
IleSerArgAlaLysTrpAsnAsnThrLeuLysGlnIleAspSerLysLeuArg
GluGlnPheGlyAsnAsnLysThrIleIlePheLysGlnSerSerGlyGlyAsp
ProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCysAsn
SerThrGlnLeuPheAsnSerThrTrpPheAsnSerThrTrpSerThrLysGly
SerAsnAsnThrGluGlySerAspThrIleThrLeuProCysArgIleLysGln
IleIleAsnMetTrpGlnGluValGlyLysAlaMetTyrAlaProProIleSer
GlyGlnIleArgCysSerSerAsnIleThrGlyLeuLeuLeuThrArgAspGly
GlyAsnSerAsnAsnGluSerGluIleArgArgGlnAlaSerArgGluLeuGlu
PheLeuLysThrLysGlyProArgAspThrProIlePheIleGly

Table 9A

Nucleotide sequence encoding fusion protein PB1

```
ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTG
TACAATGCAGGACATCTTTGGGGTTGGGCACTTTAGTTTTTTGAGCTGCCGGAC

TGGGCATTCAGTCTGGATCGCGAACGCGTGGCCGATCTGAACCAATCTGTAGAA
ACCCGTAAGTCAGACCTAGCGCTTGCGCACCGGCTAGACTTGGTTAGACATCTT

ATTAATTGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGA
TAATTAACATGTTCTGGGTTGTTGTTATGTTCTTTTTCATAGGCATAGGTCTCT

GGACCAGGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCA
CCTAATCCCTCTCGTAAACAATGTTATCCTTTTTATCCTTTATACTCTGTTCGT

CATTGTAACATTAGTAGAGCAAAATGGAATAACACTTTAAAACAGATAGATAGC
GTAACATTGTAATCATCTCGTTTTACCTTATTGTGAAATTTTGTCTATCTATCG

AAATTAAGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAGTCCTCA
TTTAATTCTCTTGTTAAACCTTTATTATTTTGTTATTAGAAATTCGTCAGGAGT

GGAGGGGACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTC
CCTCCCCTGGGTCTTTAACATTGCGTGTCAAAATTAACACCTCCCCTTAAAAAG

TACTGTAATTCAACACAACTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGT
ATGACATTAAGTTGTGTTGACAAATTATCATGAACCAAATTATCATGAACCTCA

ACTAAAGGGTCAAATAACACTGAAGGAAGTGACACAATCACCCTCCCATGCAGA
TGATTTCCCAGTTTATTGTGACTTCCTTCACTGTGTTAGTGGGAGGGTACGTCT

ATAAAACAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCCT
TATTTTGTTTAATATTTGTACACCGTCCTTCATCCTTTTCGTTACATACGGGGA

CCCATCAGTGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACA
GGGTAGTCACCTGTTTAATCTACAAGTAGTTTATAATGTCCCGACGATAATTCT

AGAGATGGTGGTAATAGCAACAATGAGTCCGAGATCCGTCGACAAGCTTCCCGG
TCTCTACCACCATTATCGTTGTTACTCAGGCTCTAGGCAGCTGTTCGAAGGGCC

GAGCTCGAATTCTTGAAGACGAAAGGGCCTCGTGATACTCCTATTTTTATAGGT
CTCGAGCTTAAGAACTTCTGCTTTCCCGGAGCACTATGCGGATAAAAATATCCA
```

Table 10

Amino acid sequence of fusion protein 590

MetLeuArgProValGluThr
ProThrArgGluIleLysLysLeuAspGlyLeuTrpAlaPheSerLeuAspArg
GluArgValAlaAspLeuAsnGlnSerValGluIleAsnCysThrArgProAsn
AsnAsnThrArgLysSerIleArgIleGlnArgGlyProGlyArgAlaPheVal
ThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCysAsnIleSerArgAla
LysTrpAsnAsnThrLeuLysGlnIleAspSerLysLeuArgGluGlnPheGly
AsnAsnLysThrIleIlePheLysGlnSerSerGlyGlyAspProGluIleVal
ThrHisSerPheAsnCysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeu
PheAsnSerThrTrpPheAsnSerThrTrpSerThrLysGlySerAsnAsnThr
GluGlySerAspThrIleThrLeuProCysArgIleLysGlnIleIleAsnMet
TrpGlnGluValGlyLysAlaMetTyrAlaProProIleSerGlyGlnIleArg
CysSerSerAsnIleThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsn
AsnGluSerGluIlePheArgProGlyGlyGlyAspMetArgAspAsnTrpArg
SerGluLeuTyrLysTyrLysValValLysIleGluProLeuGlyValAlaPro
ThrLysAlaLysArgArgValValGlnArgGluLysArgAlaValGlyIleGly
AlaLeuPheLeuGlyPheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSer
MetThrLeuThrValGlnAlaArgGlnLeuLeuSerGlyIleValGlnGlnGln
AsnAsnLeuLeuArgAlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrVal
TrpGlyIleLysGlnLeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLys
AspGlnGlnLeuLeuGlyIleTrpGlyCysSerGlyLysLeuIleCysThrThr
AlaValProTrpAsnAlaSerTrpSerAsnLysSerLeuGluGlnIleTrpAsn
AsnMetThrTrpMetGluTrpAspArgGluIleAsnAsnTyrThrSerPhePro
IleHisArgSerValMetLeuTyrThrThrProAsnThrTrpValAspAspIle
ThrValValThrHisValAlaGlnAspCysAsnHisAlaSerValAspTrpGln
ValValAlaAsnGlyAspValSerValGluLeuArgAspAlaAspGlnGlnVal

Table 10 (cont.)

ValAlaThrGlyGlnGlyThrSerGlyThrLeuGlnValValAsnProHisLeu
TrpGlnProGlyGluGlyTyrLeuTyrGluLeuCysValThrAlaLysSerGln
ThrGluCysAspIleTyrProLeuArgValGlyIleArgSerValAlaValLys
GlyGluGlnPheLeuIleAsnHisLysProPheTyrPheThrGlyPheGlyArg
HisGluAspAlaAspLeuArgGlyLysGlyPheAspAsnValLeuMetValHis
AspHisAlaLeuMetAspTrpIleGlyAlaAsnSerTyrArgThrSerHisTyr
ProTyrAlaGluGluMetLeuAspTrpAlaAspGluHisGlyIleValValIle
AspGluThrAlaAlaValGlyPheAsnLeuSerLeuGlyIleGlyPheGluAla
GlyAsnLysProLysGluLeuTyrSerGluGluAlaValAsnGlyGluThrGln
GlnAlaHisLeuGlnAlaIleLysGluLeuIleAlaArgAspLysAsnHisPro
SerValValMetTrpSerIleAlaAsnGluProAspThrArgProGlnGlyAla
ArgGluTyrPheAlaProLeuAlaGluAlaThrArgLysLeuAspProThrArg
ProIleThrCysValAsnValMetPheCysAspAlaHisThrAspThrIleSer
AspLeuPheAspValLeuCysLeuAsnArgTyrTyrGlyTrpTyrValGlnSer
GlyAspLeuGluThrAlaGluLysValLeuGluLysGluLeuLeuAlaTrpGln
GluLysLeuHisGlnProIleIleIleThrGluTyrGlyValAspThrLeuAla
GlyLeuHisSerMetTyrThrAspMetTrpSerGluGluTyrGlnCysAlaTrp
LeuAspMetTyrHisArgValPheAspArgValSerAlaValValGlyGluGln
ValTrpAsnPheAlaAspPheAlaThrSerGlnGlyIleLeuArgValGlyGly
AsnLysLysGlyIlePheThrArgAspArgLysProLysSerAlaAlaPheLeu
LeuGlnLysArgTrpThrGlyMetAsnPheGlyGluLysProGlnGlnGlyGly
LysGln

Table 10A

Nucleotide sequence encoding fusion protein 590

ATGTTACGTCCTGTAGAAACC
TACAATGCAGGACATCTTTGG

CCAACCCGTGAAATCAAAAAACTCGACGGCCTGTGGGCATTCAGTCTGGATCGC
GGTTGGGCACTTTAGTTTTTTGAGATGCCGGACACCCGTAAGTCAGACCTAGCG

GAACGCGTGGCCGATCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAAC
CTTGCGCACCGGCTAGACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTG

AACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTT
TTGTTATGTTCTTTTTCATAGGCATAGGTCTCTCCTAATCCCTCTCGTAAACAA

ACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCA
TGTTATCCTTTTTATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGT

AAATGGAATAACACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGA
TTTACCTTATTGTGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCT

AATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTA
TTATTATTTTGTTATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACAT

ACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTG
TGCGTGTCAAAATTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGAC

TTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACT
AAATTATCATGAACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGA

GAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATG
CTTCCTTCACTGTGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTAC

TGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGA
ACCGTCCTTCATCCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCT

TGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAAC
ACAAGTAGTTTATAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTG

AATGAGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGA
TTACTCAGGCTCTAGAAGTCTGGACCTCCTCCTCTATACTCCCTCTTAACCTCT

AGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCC
TCACTTAATATATTTATATTTCATCATTTTTAACTTGGTAATCCTCATCGTGGG

ACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGA
TGGTTCCGTTTCTCTTCTCACCACGTCTCTCTTTTTTCTCGTCACCCTTATCCT

Table 10A (cont.)

GCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCA
CGAAACAAGGAACCCAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGT

ATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAG
TACTGCGACTGCCATGTCCGGTCTGTTAATAACAGACCATATCACGTCGTCGTC

AACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTC
TTGTTAAACGACTCCCGATAACTCCGCGTTGTCGTAGACAACGTTGAGTGTCAG

TGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAG
ACCCCGTAGTTCGTCGAGGTCCGTTCTTAGGACCGACACCTTTCTATGGATTTC

GATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACT
CTAGTTGTCGAGGACCCCTAAACCCCAACGAGACCTTTTGAGTAAACGTGGTGA

GCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAAT
CGACACGGAACCTTACGATCAACCTCATTATTTAGAGACCTTGTCTAAACCTTA

AACATGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTCCCG
TTGTACTGGACCTACCTCACCCTGTCTCTTTAATTGTTAATGTGTTCGAAGGGC

ATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTGGACGATATC
TAGGTAGCGTCGCATTACGAGATGTGGTGCGGCTTGTGGACCCACCTGCTATAG

ACCGTGGTGACGCATGTCGCGCAAGACTGTAACCACGCGTCTGTTGACTGGCAG
TGGCACCACTGCGTACAGCGCGTTCTGACATTGGTGCGCAGACAACTGACCGTC

GTGGTGGCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCGGATCAACAGGTG
CACCACCGGTTACCACTACAGTCGCAACTTGACGCACTACGCCTAGTTGTCCAC

GTTGCAACTGGACAAGGCACTAGCGGGACTTTGCAAGTGGTGAATCCGCACCTC
CAACGTTGACCTGTTCCGTGATCGCCCTGAAACGTTCACCACTTAGGCGTGGAG

TGGCAACCGGGTGAAGGTTATCTCTATGAACTGTGCGTCACAGCCAAAAGCCAG
ACCGTTGGCCCACTTCCAATAGAGATACTTGACACGCAGTGTCGGTTTTCGGTC

ACAGAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAAG
TGTCTCACACTATAGATGGGCGAAGCGCAGCCGTAGGCCAGTCACCGTCACTTC

GGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGT
CCGCTTGTCAAGGACTAATTGGTGTTTGGCAAGATGAAATGACCGAAACCAGCA

CATGAAGATGCGGACTTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCAC
GCACTTCTACGCCTGAACGCACCGTTTCCTAAGCTATTGCACGACTACCACGTG

GACCACGCATTAATGGACTGGATTGGGGCCAACTCCTACCGTACCTCGCATTAC
CTGGTGCGTAATTACCTGACCTAACCCCGGTTGAGGATGGCATGGAGCGTAATG

Table 10A (cont.)

CCTTACGCTGAAGAGATGCTCGACTGGGCAGATGAACATGGCATCGTGGTGATT
GGAATGCGACTTCTCTACGAGCTGACCCGTCTACTTGTACCGTAGCACCACTAA

GATGAAACTGCTGCTGTCGGCTTTAACCTCTCTTTAGGCATTGGTTTCGAAGCG
CTACTTTGACGACGACAGCCGAAATTGGAGAGAAATCCGTAACCAAAGCTTCGC

GGCAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAG
CCGTTGTTCGGCTTTCTTGACATGTCGCTTCTCCGTCAGTTGCCCCTTTGAGTC

CAAGCGCACTTACAGGCGATTAAAGAGCTGATAGCGCGTGACAAAAACCACCCA
GTTCGCGTGAATGTCCGCTAATTTCTCGACTATCGCGCACTGTTTTTGGTGGGT

AGCGTGGTGATGTGGAGTATTGCCAACGAACCGGATACCCGTCCGCAAGGTGCA
TCGCACCACTACACCTCATAACGGTTGCTTGGCCTATGGGCAGGCGTTCCACGT

CGGGAATATTTCGCGCCACTGGCGGAAGCAACGCGTAAACTCGACCCGACGCGT
GCCCTTATAAAGCGCGGTGACCGCCTTCGTTGCGCATTTGAGCTGGGCTGCGCA

CCGATCACCTGCGTCAATGTAATGTTCTGCGACGCTCACACCGATACCATCAGC
GGCTAGTGGACGCAGTTACATTACAAGACGCTGCGAGTGTGGCTATGGTAGTCG

GATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGGATGGTATGTCCAAAGC
CTAGAGAAACTACACGACACGGACTTGGCAATAATGCCTACCATACAGGTTTCG

GGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGAACTTCTGGCCTGGCAG
CCGCTAAACCTTTGCCGTCTCTTCCATGACCTTTTTCTTGAAGACCGGACCGTC

GAGAAACTGCATCAGCCGATTATCATCACCGAATACGGCGTGGATACGTTAGCC
CTCTTTGACGTAGTCGGCTAATAGTAGTGGCTTATGCCGCACCTATGCAATCGG

GGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCATGG
CCCGACGTGAGTTACATGTGGCTGTACACCTCACTTCTCATAGTCACACGTACC

CTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAG
GACCTATACATAGTGGCGCAGAAACTAGCGCAGTCGCGGCAGCAGCCACTTGTC

GTATGGAATTTCGCCGATTTTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGT
CATACCTTAAAGCGGCTAAAACGCTGGAGCGTTCCGTATAACGCGCAACCGCCA

AACAAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCGGCGGCTTTTCTG
TTGTTCTTTCCCTAGAAGTGAGCGCTGGCGTTTGGCTTCAGCCGCCGAAAAGAC

CTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGCAGCAGGGAGGC
GACGTTTTTGCGACCTGACCGTACTTGAAGCCACTTTTTGGCGTCGTCCCTCCG

AAACAA
TTTGTT

Table 11

Amino acid sequence of fusion protein KH1

MetLeuArg
ProValGluThrProThrArgGluIleLysLysLeuAspGlyLeuTrpAlaPhe
SerLeuAspArgGluArgGluPheProValTrpLysGluAlaThrThrThrLeu
PheCysAlaSerAspAlaLysAlaTyrAspThrGluValHisAsnValTrpAla
ThrHisAlaCysValProThrAspProAsnProGlnGluValValLeuValAsn
ValThrGluAsnPheAsnMetTrpLysAsnAspMetValGluGlnMetHisGlu
AspIleIleSerLeuTrpAspGlnSerLeuLysProCysValLysLeuThrPro
LeuCysValSerLeuLysCysThrAspLeuLysAsnAspThrAsnThrAsnSer
SerSerGlyArgMetIleMetGluLysGlyGluIleLysAsnCysSerPheAsn
IleSerThrSerIleArgGlyLysValGlnLysGluTyrAlaPhePheTyrLys
LeuAspIleIleProIleAspAsnAspThrThrSerTyrThrLeuThrSerCys
AsnThrSerValIleThrGlnAlaCysProLysValSerPheGluProIlePro
IleHisTyrCysAlaProAlaGlyPheAlaIleLeuLysCysAsnAsnLysThr
PheAsnGlyThrGlyProCysThrAsnValSerThrValGlnCysThrHisGly
IleArgProValValSerThrGlnLeuLeuLeuAsnGlySerLeuAlaGluGlu
GluValValIleArgSerAlaAsnPheThrAspAsnAlaLysThrIleIleVal
GlnLeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArg
LysSerIleArgIleGlnArgGlyProGlyArgAlaPheValThrIleGlyLys
IleGlyAsnMetArgGlnAlaHisCysAsnIleSerArgAlaLysTrpAsnAsn
ThrLeuLysGlnIleAspSerLysLeuArgGluGlnPheGlyAsnAsnLysThr
IleIlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPhe
AsnCysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSerThr
TrpPheAsnSerThrTrpSerThrLysGlySerAsnAsnThrGluGlySerAsp
ThrIleThrLeuProCysArgIleLysGlnIleIleAsnMetTrpGlnGluVal

Table 11 (cont.)

GlyLysAlaMetTyrAlaProProIleSerGlyGlnIleArgCysSerSerAsn
IleThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsnAsnGluSerGlu
IlePheArgProGlyGlyGlyAspMetArgAspAsnTrpArgSerGluLeuTyr
LysTyrLysValValLysIleGluProLeuGlyValAlaProThrLysAlaLys
ArgArgValValGlnArgGluLysArgAlaValGlyIleGlyAlaLeuPheLeu
GlyPheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSerMetThrLeuThr
ValGlnAlaArgGlnLeuLeuSerGlyIleValGlnGlnGlnAsnAsnLeuLeu
ArgAlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLys
GlnLeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeu
LeuGlyIleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrp
AsnAlaSerTrpSerAsnLysSerLeuGluGlnIleTrpAsnAsnMetThrTrp
MetGluTrpAspArgGluIleAsnAsnTyrThrSerPheProGlyAlaArgIle
LeuGluAspGluArgAlaSer

Table 11A

Nucleotide sequence encoding fusion protein KH1

```
                                                                ATGTTACGT
                                                                TACAATGCA

CCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTGTGGGCATTC
GGACATCTTTGGGGTTGGGCACTTTAGTTTTTTGAGCTGCCGGACACCCGTAAG

AGTCTGGATCGCGAACGCGAATTCCCTGTGTGGAAGGAAGCAACCACCACTCTA
TCAGACCTAGCGCTTGCGCTTAAGGGACACACCTTCCTTCGTTGGTGGTGAGAT

TTTTGTGCATCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCC
AAAACACGTAGTCTACGATTTCGTATACTATGTCTCCATGTATTACAAACCCGG

ACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTAGTATTGGTAAAT
TGTGTACGGACACATGGGTGTCTGGGGTTGGGTGTTCTTCATCATAACCATTTA

GTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAACAGATGCATGAG
CACTGTCTTTTAAAATTGTACACCTTTTTACTGTACCATCTTGTCTACGTACTC

GATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCA
CTATATTAGTCAAATACCCTAGTTTCGGATTTCGGTACACATTTTAATTGGGGT

CTCTGTGTTAGTTTAAAGTGCACTGATTTGAAGAATGATACTAATACCAATAGT
GAGACACAATCAAATTTCACGTGACTAAACTTCTTACTATGATTATGGTTATCA

AGTAGCGGGAGAATGATAATGGAGAAAGGAGAGATAAAAAACTGCTCTTTCAAT
TCATCGCCCTCTTACTATTACCTCTTTCCTCTCTATTTTTTGACGAGAAAGTTA

ATCAGCACAAGCATAAGAGGTAAGGTGCAGAAAGAATATGCATTTTTTTATAAA
TAGTCGTGTTCGTATTCTCCATTCCACGTCTTTCTTATACGTAAAAAAATATTT

CTTGATATAATACCAATAGATAATGATACTACCAGCTATACGTTGACAAGTTGT
GAACTATATTATGGTTATCTATTACTATGATGGTCGATATGCAACTGTTCAACA

AACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCAATTCCC
TTGTGGAGTCAGTAATGTGTCCGGACAGGTTTCCATAGGAAACTCGGTTAAGGG

ATACATTATTGTGCCCCCGGCTGGTTTTGCGATTCTAAAATGTAATAATAAGACG
TATGTAATAACACGGGGCCGACCAAAACGCTAAGATTTTACATTATTATTCTGC

TTCAATGGAACAGGACCATGTACAAATGTCAGCACAGTACAATGTACACATGGA
AAGTTACCTTGTCCTGGTACATGTTTACAGTCGTGTCATGTTACATGTGTACCT

ATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAATGGCAGTCTAGCAGAAGAA
TAATCCGGTCATCATAGTTGAGTTGACGACAATTTACCGTCAGATCGTCTTCTT

GAGGTAGTAATTAGATCTGCCAATTTCACAGACAATGCTAAAACCATAATAGTA
CTCCATCATTAATCTAGACGGTTAAAGTGTCTGTTACGATTTTGGTATTATCAT
```

Table 11A (cont.)

CAGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGA
GTCGACTTGGTTAGACATCTTTAATTAACATGTTCTGGGTTGTTGTTATGTTCT

AAAAGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTTACAATAGGAAAA
TTTTCATAGGCATAGGTCTCTCCTGGTCCCTCTCGTAAACAATGTTATCCTTTT

ATAGGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATAAC
TATCCTTTATACTCTGTTCGTGTAACATTGTAATCATCTCGTTTTACCTTATTG

ACTTTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAATAAAACA
TGAAATTTTGTCTATCTATCGTTTAATTCTCTTGTTAAACCTTTATTATTTTGT

ATAATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTT
TATTAGAAATTCGTCAGGAGTCCTCCCCTGGGTCTTTAACATTGCGTGTCAAAA

AATTGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGTACT
TTAACACCTCCCCTTAAAAAGATGACATTAAGTTGTGTTGACAAATTATCATGA

TGGTTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAGTGAC
ACCAAATTATCATGAACCTCATGATTTCCCAGTTTATTGTGACTTCCTTCACTG

ACAATCACCCTCCCATGCAGAATAAAACAAATTATAAACATGTGGCAGGAAGTA
TGTTAGTGGGAGGGTACGTCTTATTTTGTTTAATATTTGTACACCGTCCTTCAT

GGAAAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGATGTTCATCAAAT
CCTTTTCGTTACATACGGGGAGGGTAGTCACCTGTTTAATCTACAAGTAGTTTA

ATTACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCCGAG
TAATGTCCCGACGATAATTGTTCTCTACCACCATTATCGTTGTTACTCAGGCTC

ATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATAT
TAGAAGTCTGGACCTCCTCCTCTATACTCCCTGTTAACCTCTTCACTTAATATA

AAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAG
TTTATATTTCATCATTTTTAACTTGGTAATCCTCATCGTGGGTGGTTCCGTTTC

AGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTT
TCTTCTCACCACGTCTCTCTTTTTTCTCGTCACCCTTATCCTCGAAACAAGGAA

GGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACG
CCCAAGAACCCTCGTCGTCCTTCGTGATACCCGCGTCGCAGTTACTGCGACTGC

GTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTG
CATGTCCGGTCTGTTAATAACAGACCATATCACGTCGTCGTCTTGTTAAACGAC

AGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG
TCCCGATAACTCCGCGTTGTCGTAGACAACGTTGAGTGTCAGACCCCGTAGTTC

CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTC
GTCGAGGTCCGTTCTTAGGACCGACACCTTTCTATGGATTTCCTAGTTGTCGAG

Table 11A (cont.)

CTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGG
GACCCCTAAACCCCAACGAGACCTTTTGAGTAAACGTGGTGACGACACGGAACC

AATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATAACATGACCTGG
TTACGATCAACCTCATTATTTAGAGACCTTGTCTAAACCTTATTGTACTGGACC

ATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTCCCGGGAGCTCGAATT
TACCTCACCCTGTCTCTTTAATTGTTAATGTGTTCGAAGGGCCCTCGAGCTTAA

CTTGAAGACGAAAGGGCCTCG
GAACTTCTGCTTTCCCGGAGC

Table 12

Amino acid sequence of HIV portion of protein R10

MetValTrpLysGluAlaThrThrThrLeuPheCysAlaSerAspAlaLysAlaTyr
AspThrGluValHisAsnValTrpAlaThrHisAlaCysValProThrAspPro
AsnProGlnGluValValLeuValAsnValThrGluAsnPheAsnMetTrpLys
AsnAspMetValGluGlnMetHisGluAspIleIleSerLeuTrpAspGlnSer
LeuLysProCysValLysLeuThrProLeuCysValSerLeuLysCysThrAsp
LeuLysAsnAspThrAsnThrAsnSerSerSerGlyArgMetIleMetGluLys
GlyGluIleLysAsnCysSerPheAsnIleSerThrSerIleArgGlyLysVal
GlnLysGluTyrAlaPhePheTyrLysLeuAspIleIleProIleAspAsnAsp
ThrThrSerTyrThrLeuThrSerCysAsnThrSerValIleThrGlnAlaCys
ProLysValSerPheGluProIleProIleHisTyrCysAlaProAlaGlyPhe
AlaIleLeuLysCysAsnAsnLysThrPheAsnGlyThrGlyProCysThrAsn
ValSerThrValGlnCysThrHisGlyIleArgProValValSerThrGlnLeu
LeuLeuAsnGlySerLeuAlaGluGluGluValValIleArgSerAlaAsnPhe
ThrAspAsnAlaLysThrIleIleValGlnLeuAsnGlnSerValGluIleAsn
CysThrArgProAsnAsnAsnThrArgLysSerIleArgIleGlnArgGlyPro
GlyArgAlaPheValThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCys
AsnIleSerArgAlaLysTrpAsnAsnThrLeuLysGlnIleAspSerLysLeu
ArgGluGlnPheGlyAsnAsnLysThrIleIlePheLysGlnSerSerGlyGly
AspProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCys
AsnSerThrGlnLeuPheAsnSerThrTrpPheAsnSerThrTrpSerThrLys
GlySerAsnAsnThrGluGlySerAspThrIleThrLeuProCysArgIleLys
GlnIleIleAsnMetTrpGlnGluValGlyLysAlaMetTyrAlaProProIle
SerGlyGlnIleArgCysSerSerAsnIleThrGlyLeuLeuLeuThrArgAsp
GlyGlyAsnSerAsnAsnGluSer

Table 12A
Nucleotide sequence encoding
HIV portion of protein R10

ATGGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
AACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAA
AATGACATGGTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGC
CTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGAT
TTGAAGAATGATACTAATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAA
GGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTG
CAGAAAGAATATGCATTTTTTTTATAAACTTGATATAATACCAATAGATAATGAT
ACTACCAGCTATACGTTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGT
CCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
GCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTG
CTGTTAAATGGCAGTCTGGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTC
ACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAAT
TGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCA
GGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGT
AACATTAGTAGAGCAAAATGGAATAACACTTTAAAACAGATAGATAGCAAATTA
AGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGG
GACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGT
AATTCAACACAACTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAA
GGGTCAAATAACACTGAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAA
CAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATC
AGTGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGAT
GGTGGTAATAGCAACAATGAGTCC

Table 13

Amino acid sequence of HIV portion of protein PB1

Met LeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArgLys
SerIleArgIleGlnArgGlyProGlyArgAlaPheValThrIleGlyLysIle
GlyAsnMetArgGlnAlaHisCysAsnIleSerArgAlaLysTrpAsnAsnThr
LeuLysGlnIleAspSerLysLeuArgGluGlnPheGlyAsnAsnLysThrIle
IlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPheAsn
CysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSerThrTrp
PheAsnSerThrTrpSerThrLysGlySerAsnAsnThrGluGlySerAspThr
IleThrLeuProCysArgIleLysGlnIleIleAsnMetTrpGlnGluValGly
LysAlaMetTyrAlaProProIleSerGlyGlnIleArgCysSerSerAsnIle
ThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsnAsnGluSer

Table 13A
Nucleotide sequence encoding
HIV portion of protein PB1

ATGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAA

AGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTTACAATAGGAAAAATA

GGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATAACACT

TTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATA

ATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAAT

TGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGTACTTGG

TTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAGTGACACA

ATCACCCTCCCATGCAGAATAAAACAAATTATAAACATGTGGCAGGAAGTAGGA

AAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGATGTTCATCAAATATT

ACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCC

Table 14
Amino acid sequence of
HIV protion of protein 590

MetLeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArgLys
SerIleArgIleGlnArgGlyProGlyArgAlaPheValThrIleGlyLysIle
GlyAsnMetArgGlnAlaHisCysAsnIleSerArgAlaLysTrpAsnAsnThr
LeuLysGlnIleAspSerLysLeuArgGluGlnPheGlyAsnAsnLysThrIle
IlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPheAsn
CysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSerThrTrp
PheAsnSerThrTrpSerThrLysGlySerAsnAsnThrGluGlySerAspThr
IleThrLeuProCysArgIleLysGlnIleIleAsnMetTrpGlnGluValGly
LysAlaMetTyrAlaProProIleSerGlyGlnIleArgCysSerSerAsnIle
ThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsnAsnGluSerGluIle
PheArgProGlyGlyGlyAspMetArgAspAsnTrpArgSerGluLeuTyrLys
TyrLysValValLysIleGluProLeuGlyValAlaProThrLysAlaLysArg
ArgValValGlnArgGluLysArgAlaValGlyIleGlyAlaLeuPheLeuGly
PheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSerMetThrLeuThrVal
GlnAlaArgGlnLeuLeuSerGlyIleValGlnGlnGlnAsnAsnLeuLeuArg
AlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLysGln
LeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeuLeu
GlyIleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsn
AlaSerTrpSerAsnLysSerLeuGluGlnIleTrpAsnAsnMetThrTrpMet
GluTrpAspArgGluIleAsnAsnTyrThr

Table 14A
Nucleotide sequence encoding
HIV portion of protein 590

ATGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAA

AGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTTGTTACAATAGGAAAAATA

GGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATAACACT

TTAAAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATA

ATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAAT

TGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACTGTTTAATAGTACTTGG

TTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAGTGACACA

ATCACCCTCCCATGCAGAATAAAACAAATTATAAACATGTGGCAGGAAGTAGGA

AAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGATGTTCATCAAATATT

ACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCCGAGATC

TTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAA

TATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGA

AGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGG

TTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTA

CAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGG

GCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAG

CTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTG

GGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAAT

GCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATAACATGACCTGGATG

GAGTGGGACAGAGAAATTAACAATTACACA

Table 15

Amino acid sequence of

HIV portion of protein KH1

Met ValTrpLysGluAlaThrThrThrLeuPheCysAlaSerAspAlaLysAlaTyr

AspThrGluValHisAsnValTrpAlaThrHisAlaCysValProThrAspPro

AsnProGlnGluValValLeuValAsnValThrGluAsnPheAsnMetTrpLys

AsnAspMetValGluGlnMetHisGluAspIleIleSerLeuTrpAspGlnSer

LeuLysProCysValLysLeuThrProLeuCysValSerLeuLysCysThrAsp

LeuLysAsnAspThrAsnThrAsnSerSerSerGlyArgMetIleMetGluLys

GlyGluIleLysAsnCysSerPheAsnIleSerThrSerIleArgGlyLysVal

GlnLysGluTyrAlaPhePheTyrLysLeuAspIleIleProIleAspAsnAsp

ThrThrSerTyrThrLeuThrSerCysAsnThrSerValIleThrGlnAlaCys

ProLysValSerPheGluProIleProIleHisTyrCysAlaProAlaGlyPhe

AlaIleLeuLysCysAsnAsnLysThrPheAsnGlyThrGlyProCysThrAsn

ValSerThrValGlnCysThrHisGlyIleArgProValValSerThrGlnLeu

LeuLeuAsnGlySerLeuAlaGluGluGluValValIleArgSerAlaAsnPhe

ThrAspAsnAlaLysThrIleIleValGlnLeuAsnGlnSerValGluIleAsn

CysThrArgProAsnAsnAsnThrArgLysSerIleArgIleGlnArgGlyPro

GlyArgAlaPheValThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCys

AsnIleSerArgAlaLysTrpAsnAsnThrLeuLysGlnIleAspSerLysLeu

ArgGluGlnPheGlyAsnAsnLysThrIleIlePheLysGlnSerSerGlyGly

AspProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCys

AsnSerThrGlnLeuPheAsnSerThrTrpPheAsnSerThrTrpSerThrLys

GlySerAsnAsnThrGluGlySerAspThrIleThrLeuProCysArgIleLys

GlnIleIleAsnMetTrpGlnGluValGlyLysAlaMetTyrAlaProProIle

SerGlyGlnIleArgCysSerSerAsnIleThrGlyLeuLeuLeuThrArgAsp

GlyGlyAsnSerAsnAsnGluSerGluIlePheArgProGlyGlyGlyAspMet

ArgAspAsnTrpArgSerGluLeuTyrLysTyrLysValValLysIleGluPro

Table 15 (cont.)

LeuGlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArg
AlaValGlyIleGlyAlaLeuPheLeuGlyPheLeuGlyAlaAlaGlySerThr
MetGlyAlaAlaSerMetThrLeuThrValGlnAlaArgGlnLeuLeuSerGly
IleValGlnGlnGlnAsnAsnLeuLeuArgAlaIleGluAlaGlnGlnHisLeu
LeuGlnLeuThrValTrpGlyIleLysGlnLeuGlnAlaArgIleLeuAlaVal
GluArgTyrLeuLysAspGlnGlnLeuLeuGlyIleTrpGlyCysSerGlyLys
LeuIleCysThrThrAlaValProTrpAsnAlaSerTrpSerAsnLysSerLeu
GluGlnIleTrpAsnAsnMetThrTrpMetGluTrpAspArgGluIleAsnAsn
TyrThr

Table 15A
Nucleotide sequence encoding
HIV portion of protein KH1

ATGGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
AACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAA
AATGACATGGTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGC
CTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGAT
TTGAAGAATGATACTAATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAA
GGAGAGATAAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTG
CAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGAT
ACTACCAGCTATACGTTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGT
CCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
GCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTG
CTGTTAAATGGCAGTCTGGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTC
ACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAAT
TGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCA
GGGAGAGCATTTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGT
AACATTAGTAGAGCAAAATGGAATAACACTTTAAAACAGATAGATAGCAAATTA
AGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGG
GACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGT
AATTCAACACAACTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAA
GGGTCAAATAACACTGAAGGAAGTGACACAATCACCCTCCCATGCAGAATAAAA
CAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCCTCCCATC
AGTGGACAAATTAGATGTTCATCAAATATTACAGGGCTGCTATTAACAAGAGAT

Table 15A (cont.)

GGTGGTAATAGCAACAATGAGTCCGAGATCTTCAGACCTGGAGGAGGAGATATG

AGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCA

TTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGA

GCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACT

ATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGT

ATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTG

TTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTG

GAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAA

CTCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTG

GAACAGATTTGGAATAACATGACCTGGATGGAGTGGGACAGAGAAATTAACAAT

TACACA

The claims defining the invention are as follows:

1. A process for stimulating a lymphocyte proliferative response in humans which comprises treating humans in need of stimulation of a lymphocyte proliferative response with a recombinant HIV portion of an HTLV-III protein selected from the group consisting of R10, PB1, 590 and KH1, wherein members of said group are as hereinbefore described.

2. A process, according to claim 1, wherein said recombinant HIV portion is the HTLV-III protein portion of R10.

3. A process, according to claim 1, wherein said recombinant HIV portion is the HTLV-III protein portion of PB1.

4. A process, according to claim 1, wherein said recombinant HIV portion is the HTLV-III protein portion of 590.

5. A process, according to claim 1, wherein said recombinant HIV portion is the HTLV-III protein portion of KH1.

DATED this SEVENTH day of MARCH 1991

Repligen Corporation

Patent Attorneys for the Applicant
SPRUSON & FERGUSON

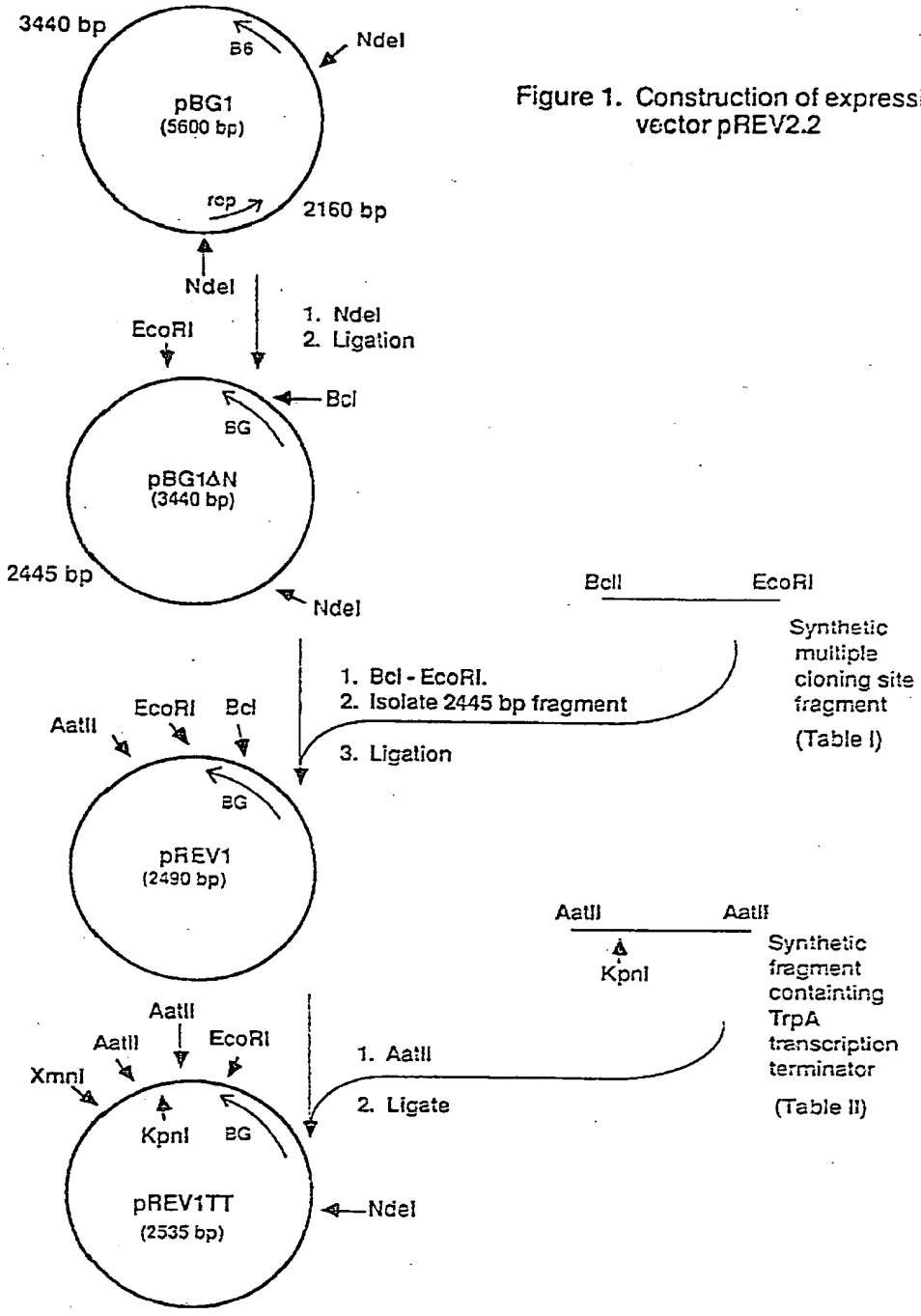
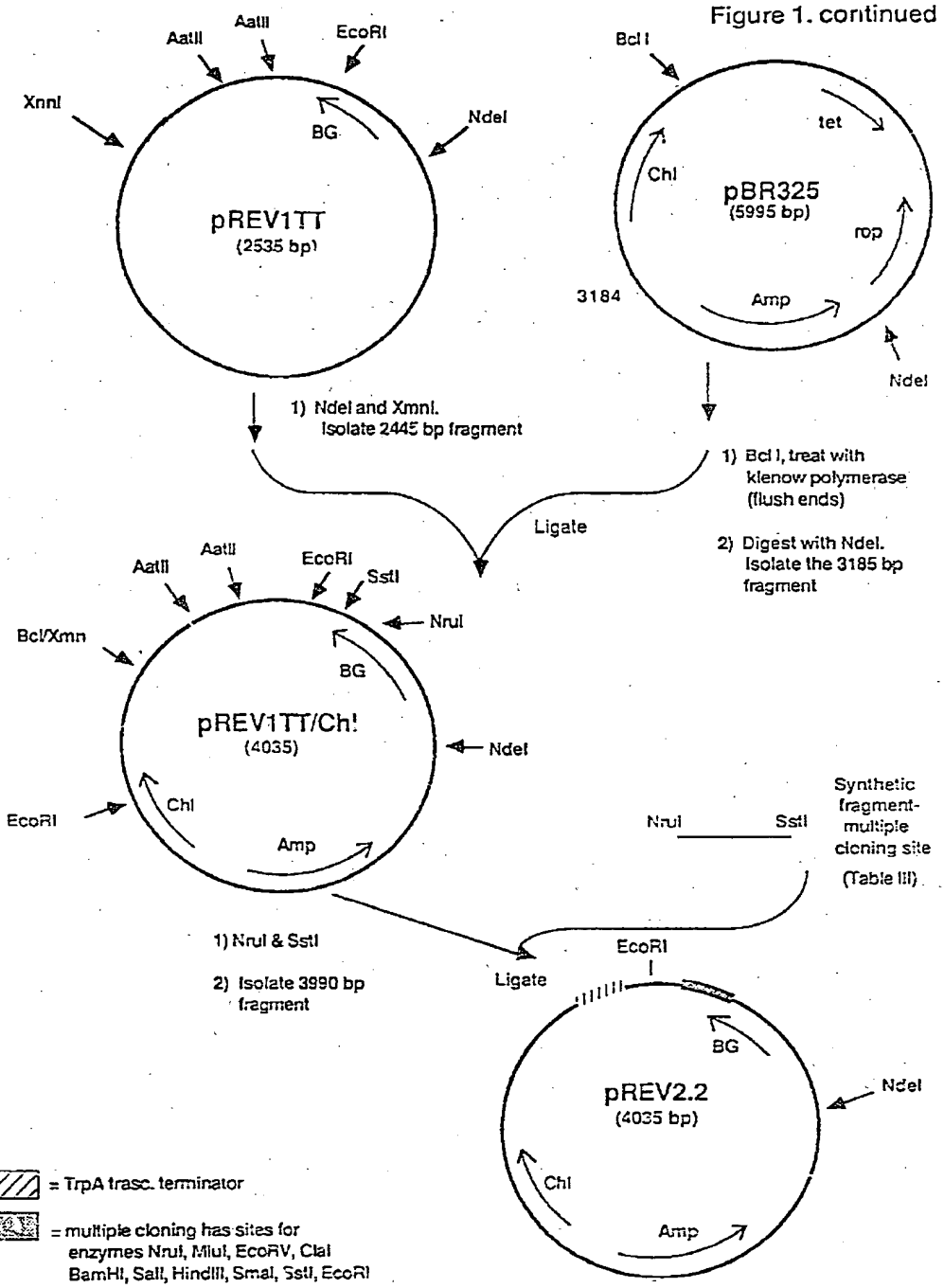


Figure 1. Construction of expression vector pREV2.2

Figure 1. continued



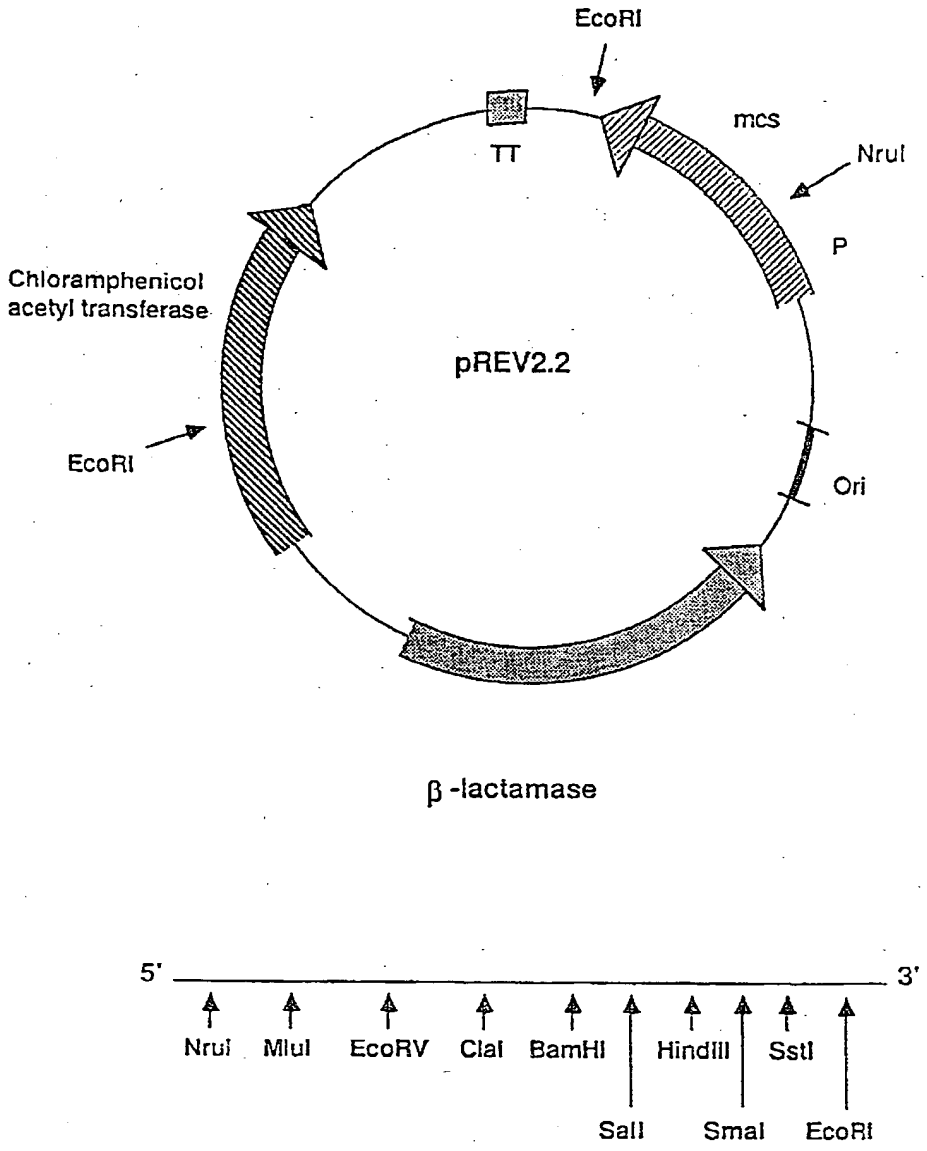


Figure 2. Schematic of pREV2.2 and of Multiple Cloning Site

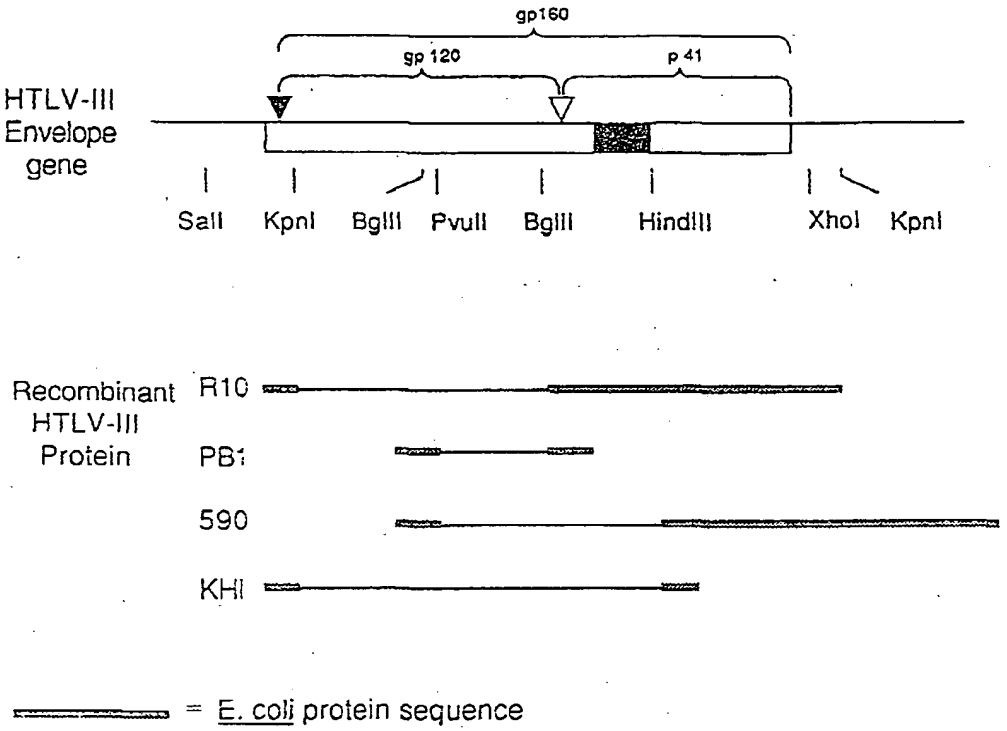
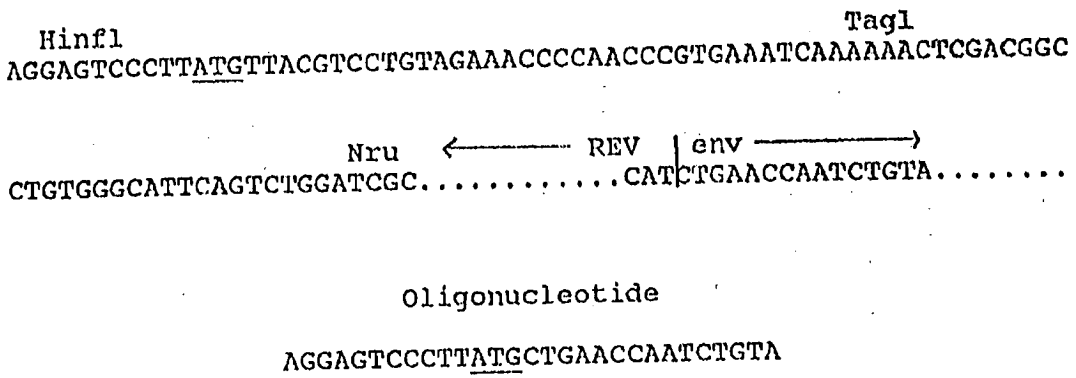


Figure 3. Schematic of HTLV-III envelope gene and recombinant proteins obtained therefrom.

FIGURE 4

Removal of N-Terminal Non-HIV Sequences of PB1

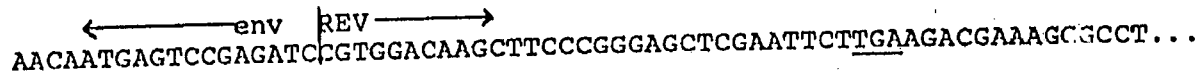


Oligonucleotide

AGGAGTCCCTTATGCTGAACCAATCTGTA

FIGURE 5

Removal of C-Terminal Non-HIV Sequences from PB1



Oligonucleotide

AACAATGAGTCCGAGATCTGAAGACGAAAGGGCCTCGTG